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STUDIES ON BOVINE MASTITIS.

III. THE DIAGNOSIS OF STREPTOCOCCUS MASTITIS.

By F. C. MINETT, A. W. STABLEFORTH, and S. J. EDWARDS,

Research Institute in Animal Pathology, Royal Veterinary College, London.

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It is well recognised that infection of the mammary gland with pathogenic bacteria can exist without giving rise to obvious pathological changes in the gland itself or alteration in the characters of the milk secreted. In such cases it is frequently impossible to make a diagnosis by clinical means, and recourse must be had to the laboratory. Attention has been chiefly directed towards the widely prevalent form of mastitis, chronic in its course, and of occult nature, which is almost invariably caused by streptococci. In a previous paper by Minett, Stableforth, and Edwards (1929), strains of mastitis streptococci were arranged primarily according to their hæmolytic effects in ox blood agar plates, a non-hæmolytic group being further divided for purposes of description into subgroups I and II. In our experience the more chronic and occult forms of mastitis, with which this paper is mainly concerned, are caused by hæmolytic streptococci and by non-hæmolytic streptococci belonging to subgroup I. It is now apparent that non-hæmolytic streptococci of subgroup II produce a form of mastitis which tends to take a more acute course, and which in consequence can often be diagnosed without laboratory assistance.

Of the methods which have been put forward for the laboratory diagnosis of chronic streptococcus mastitis, the following are discussed in this work : (*a*) cultural examination of the milk ; (*b*) microscopic examination of the centrifuge deposit or of the cream ; (*c*) estimation of the amount of sediment obtained by centrifuging a given volume of milk ; (*d*) estimation of the reaction of the milk. Still other methods have been proposed, such as the chloride figure, alcohol, and catalase tests, but no study has been made of these, since, with the possible exception of the last-named test, there is no good evidence for supposing that they possess any special advantage.

In our work the same samples of milk were submitted to examination by the methods of diagnosis under investigation, in order that their values might be compared. To reduce outside contamination during the taking of samples, elaborate precautions, such as washing of the teats and sponging with alcohol, are not required. It is sufficient to wipe the udder and teats thoroughly with a wet cloth and to reject the first stream of milk from each teat. About 15 c.c. of the fore milk (see later, page 168) from each quarter were then drawn into a sterile tube which was immediately corked and put in a cool place until all the samples had been collected. In many cases a mixed sample of milk from the udder—about 4 c.c. from each quarter—was taken. The tubes were then carried to the laboratory and placed at a temperature varying from 2° to 10° C. until the next day. Especially during the warm weather, trouble may be experienced from moulds or from spreading contamination on the surface of the culture medium. This can be largely obviated by ensuring that the udder and teats are thoroughly wetted beforehand and by cooling the samples as soon as they are collected. The samples should not be taken during the drying-off period, nor less than seven days after calving (see later, page 177).

The examination was made according to the following general

plan, the details of which will be described later under the appropriate heading. After the contents of the tubes had been thoroughly mixed, the naked eye characters of the milk were recorded, and a portion was removed for estimating the reaction to brom cresol purple or in certain cases for the preparation of smears of gravity cream. Ten c.c. were then centrifuged in a special tube, and the volume and characters of the sediment noted. The supernatant milk, with the exception of about 0·25 c.c., was then rejected, a loopful of the deposit was withdrawn for the purpose of making a smear, and the remainder was plated in blood agar. From this colonies were picked and identified when considered necessary. In certain cases a portion of the deposit was transferred to a fluid selective medium containing glucose.

Work on the subject of diagnosis was begun in June, 1928, when quarter samples were taken at the afternoon's milking weekly for a period of about three months from a group of twenty cows, about half of which were infected in at least one quarter with mastitis streptococci of the hæmolytic type. The history of these cows was obtained so far as it was available, and at intervals of about a month the udders were examined clinically. The herd was situated seven miles from the laboratory, so that about three hours elapsed before the first samples taken could be placed in really cool surroundings. During 1929 three cows (Nos. 1, 2 and 3) harbouring hæmolytic streptococci in their udders were kept at the Institute, in order that a more intensive examination of their udders might be made and samples might be examined as soon as they were taken. Similarly, during 1930, three cows (Nos. 4, 5 and 6) affected with mastitis due to non-hæmolytic streptococci were kept under close observation at the Institute for the same purpose. In addition, since 1928, about 7,000 samples from 480 cows have been examined, the samples being derived from quarters which were healthy or infected with mastitis streptococci of the hæmolytic or non-hæmolytic types. Mixed samples from many of these cows were also examined at intervals. Except in the case of mixed samples, the details of this work are not included in this paper, but it can be stated that the general results are in conformity with the conclusions set out below.

Methods of Diagnosis Investigated.

(*a*) Cultural Examination of the Milk.

This method is considered first because it aims at direct demonstration of the causal organism, and because, like other workers, we have found that a larger proportion of streptococcus-infected cows can be picked out by cultural examination than in any other way. Cultural examination can therefore be used as a basis for assessing the diagnostic value of other procedures.

Technique.

With regard to the culture medium, some workers have used plain agar or serum agar, while others have used special media designed to have

a selective action for mastitis streptococci. According to Seelemann (1928), surface colonies of mastitis streptococci on agar are sufficiently characteristic for diagnosis, but, considering these streptococci as a whole, in our experience more than one type of colony exists. In a recent article Diernhofer (1930) has given a good example showing the necessity of a somewhat more specialised medium, such as blood agar, for distinguishing two varieties of streptococci in the same sample of secretion. Klimmer, Haupt, and Roots (1928) recommend that the material should be spread on a special alkali-albuminate agar medium containing saccharose and brom cresol purple, on which colonies of mastitis streptococci produce a yellow coloration.

In our work extensive use has been made of blood agar poured plates, as these have several advantages. Not only is blood agar a rich medium which is eminently adapted for the cultivation of streptococci, but it is also to some extent selective, since it enables a primary division to be made into hæmolytic and non-hæmolytic colonies. Deep colonies of hæmolytic streptococci with their clear haloes stand out prominently, and, even when present in small numbers, are readily detected, and can be distinguished without difficulty from non-hæmolytic colonies, such as those of some varieties of staphylococci which occur normally in the cow's udder. Also, the peculiar " alpha " type of hæmolysis (Brown, 1919) which is shown by some mastitis streptococci can be distinguished in this medium. Apart from, or perhaps on account of, possible pathological significance attaching to the terms " hæmolytic " and " non-hæmolytic," these expressions are now so widely used in the literature descriptions of streptococci that it is desirable to know the effect of any given strain upon blood. If this can be ascertained during the process of isolation there is a saving in time and effort.

Deep cultivation in blood agar should be used instead of surface cultivation for several reasons. Firstly, the distinction of hæmolytic type can be made accurately only by observing deep colonies ; secondly, the number of colonies can be estimated more exactly ; and, thirdly, it is a point of practical importance that with deep cultivation a diagnosis can often be made in spite of spreading surface contamination. Finally, from the point of view of diagnosis of mastitis in general a blood agar medium is useful, especially for the recognition of *B. pyogenes*, which produces hæmolytic colonies after forty-eight hours' incubation.

In order to determine whether it was preferable to use centrifuge deposit or cream as seed material for sowing plates, milk samples from twenty quarters harbouring hæmolytic streptococci were examined. Ten c.c. of each sample were centrifuged, and the whole deposit, varying from less than 0·01 to 0·04 c.c., was plated. Another portion of each sample was allowed to stand for two hours, and a large loopful (approximately 0·02 c.c.) of cream, which is about the most that can be satisfactorily plated, was used for making dilutions. In every instance larger numbers of streptococci could be cultivated from the deposit than from the cream, the difference being far greater than could be accounted for by divergences in the amount of material used. Considering the counts on the second plates in each series,

the differences on an average were of the order of thirty to one in favour of the deposits. Under the conditions, therefore, in which a comparison has to be made, deposits are to be preferred to cream for plating. In cases where the milk sample was practically normal in appearance the centrifuge deposit was mixed with about 2 c.c. normal saline, of which one-half was plated directly in 10 c.c. agar containing 5 per cent. defibrinated ox blood. The remaining half was further diluted in 9 c.c. saline, of which 1 c.c. was similarly plated. After twenty hours, and again after forty-four to forty-eight hours, at 37° C., the plates were examined, and the type and approximate number of colonies noted. When the milk samples were obviously altered in appearance the two saline dilutions were made from a small quantity, usually about 0·1 c.c., of the uncentrifuged material.

After some experience, a mere glance at the plate is sufficient in many cases to show whether streptococcus colonies are present or whether the colonies are those of normal udder bacteria. The latter, for the most part, consist of staphylococci, deep colonies being non-hæmolytic, though occasionally a certain amount of hæmolysis may be produced. Colonies of these organisms, however, after twenty-four hours' incubation, are usually larger than streptococcus colonies, and the zone of hæmolysis is smaller.

As a rule there is no difficulty in recognising colonies of hæmolytic streptococci, even when they are present in small numbers. Deep colonies at the twentieth hour are surrounded by a clear zone in which hæmolysis is complete. Should there be any doubt as to their nature, two or three colonies may be picked to tubes of broth, and the resulting cultures examined microscopically after overnight incubation. The identification may be carried further by transferring some of the growth to litmus milk, which is rapidly acidified with coagulation, and to other test media such as 1 per cent. glucose broth or hippurate broth.

Whereas the detection of hæmolytic streptococci in blood agar seeded with milk sediments presents no great difficulty, the recognition of non-hæmolytic mastitis streptococci in similar circumstances is not always so simple. When these are present in large numbers the more heavily seeded plates show after overnight incubation very numerous tiny colonies which are surrounded by a greenish zone of variable intensity. After forty-eight hours at 37° C., examination under a low power of the microscope shows that the deep colonies on the more crowded plates are still small, and that many of them have assumed a definite " alpha " appearance. A little experience will enable deep colonies of streptococci to be distinguished with small risk of error, and the appearance of surface colonies will provide useful confirmatory evidence.

When non-hæmolytic streptococci are present in small numbers only diagnosis is more difficult, because the alpha appearance is likely to be absent and the colonies cannot be readily distinguished from those of staphylococci. In such cases it has been found useful to sow a portion of the centrifuged milk deposit into a fluid selective

medium containing glucose, details of which will be published later, and after overnight incubation to examine smears of the bottom growth for streptococci. If chains of streptococci are seen isolation and identification are indicated.

When the cultural examination is confined to blood agar plating, it is essential to pick representative colonies to broth, and if these prove to be streptococci to transfer them to various test media, in order to determine to which group of non-hæmolytic udder streptococci they belong. This is a subject which will be dealt with later by one of us. Of the test substances referred to it is only necessary at the moment to mention mannite. It is certain that as a rule non-hæmolytic streptococci associated with mastitis do not ferment this substance, but we have recently encountered a few cases of mastitis in which mannite-fermenting streptococci were present in the secretion in overwhelming numbers, and it therefore seems highly probable that they were the cause.

Influence of Certain Variations of Technique upon the Streptococcus Count.

Among the factors which may have an influence upon the streptococcus count of milk may be mentioned :—

i. The stage during milking at which the sample is taken.
ii. The time elapsing and conditions of storage before plating.
iii. The actual technique of plating, including such factors as age and temperature of the defibrinated blood used, and the interval between the preparing of milk dilutions and the addition of blood or the pouring of the agar.

Before making observations on the possible influence of these factors it was necessary to be assured that there were no important variations in the numbers of colonies growing on different plates set up from the same dilution. For this purpose three to six sets of blood agar plates were made from suitable serial dilutions of each of four samples of milk which were known to contain easily recognisable types of streptococci. In one experiment a single series of dilutions was made from each milk sample and one plate then prepared from each dilution. It was found that the counts from the same dilution did not show, as a rule, more than a 10 per cent. variation, the greatest variation being about 20 per cent.

i. *Stage during milking at which the sample is taken.*—Klimmer and Haupt (1930), in a recent paper, express the view that as a general rule it is preferable to carry out cultural examination with samples of the mid milk. In some cases of mastitis the characters of the milk —and more especially of the fore milk—are greatly changed, and it may then be impossible to cultivate streptococci although these organisms are visible microscopically. This occasional failure to cultivate streptococci from the greatly altered secretion has been noted by several workers, and the reasons which have been put forward do not appear to be very satisfactory.

Our results indicate that from the point of view of the numbers

of streptococci present, and in cases at any rate where the milk is not much changed in appearance, it is advantageous to examine the fore milk. In investigating this point samples were taken from the fore, mid, and strip milk of twenty infected quarters and plated out n similar dilutions in blood agar. In eleven cases the streptococcus count was highest in the fore milk, and in two of these in which the counts were low streptococcus colonies were present only in the fore milk plates. In five cases the count was highest with the strip milk, and in three cases highest with the mid milk.

ii. *Influence of storage.* Davis and Capps (1914) found that storage at laboratory temperature for periods up to six hours had little effect on the streptococcus count. On the other hand, Rullman and Trommsdorff (1906) found that in some cases during the first five to seven hours after milking there was a distinct drop in the bacterial count of milk stored at temperatures between 15° and 25° C.

Fore milk samples from each of five infected quarters were plated in blood agar, after being treated as follows :—

(*a*) Cooled to 2° C., a process occupying about four hours, after which the sample was allowed to return to laboratory temperature.

(*b*) Placed in an ice chest at 2° C. for twenty-eight hours.

(*c*) Stored at 20° to 21° C. for five hours.

(*d*) Stored at 20° to 21° C. for five hours, and then placed at 2° C. for twenty-four hours.

A sample from each quarter was plated immediately it was drawn as a control. The results showed that on the whole the variation in count produced by variations in methods of storage was not striking.

With samples stored as (*c*) and (*d*), however, the count was generally found to have dropped slightly, and in a few cases the fall reached 50 or 60 per cent.

iii. *Technique of plating.* Experiments were made to determine the effect upon the count of :—

(*a*) Allowing the diluted milk to remain in the plates for periods up to six hours before adding the medium ;

(*b*) Leaving defibrinated blood, freshly taken from the animal or after storage for a week in the cold, in contact with the diluted milk for periods up to six hours before adding the agar ;

(*c*) Using cold blood, *i.e.*, within twenty minutes of being removed from the cold chest.

It was found that there were no important variations in the count unless the milk dilutions were left in contact with the blood—fresh or up to one week old—for at least three hours before adding the agar. In this case the counts were distinctly lower. Chilled blood, however, produced a definite lag, and hæmolytic colonies were often poorly developed and greenish.

Cultural Examination of Milk Samples from Three Series of Infected Cows.

Series I comprised the twenty cows referred to above, quarter samples from which were examined weekly for three months. Of

these cows about one-half were harbouring hæmolytic streptococci in one or more quarters of the udder, and several of them suffered from clinical mastitis before or during the three months period of observation. With this series, dilutions of whole milk corresponding to 0·1 and 0·01 c.c. milk were plated in order that the numbers of streptococci present at successive examinations might be compared.

In this way thirty infected quarters were examined, and the most striking fact noted was the great variation in the number of streptococci excreted from the same quarter from week to week. Frequently innumerable colonies developed on the plate seeded with 0·01 c.c. milk, while a week or two later the number of colonies might not exceed twenty on the plate seeded with 0·1 c.c. milk, or colonies might be absent altogether. Rises in the count to " innumerable," with much lower intervening counts, were noted during the period of observation, once in each of thirteen quarters, twice in each of ten quarters, three times in each of six quarters, while in one quarter four such rises were recorded (see Chart I). In all cases the organisms present were typical hæmolytic streptococci. This rise and fall in the count no doubt reflects the varying state of activity of the disease process, but from the point of view of diagnosis it is important to observe that even with an infected quarter the number of streptococci present in the fore milk may at times be very small, and this emphasised the desirability of using milk sediments rather than whole milk for plating.

Series II.—The experiments just mentioned were carried out during the summer of 1928. Owing to the distance of the farm from the laboratory, about three hours elapsed before the samples could be placed in cool surroundings, and consequently the laboratory examination was postponed until the next day. Although there was no reason to suppose that this delay would affect the results in an important degree, and although a period of three hours would often be greatly exceeded in practice, it was advisable to be assured that the variation in streptococcus count was not due to some extent to the delay before plating. For this and other reasons, during 1929, three cows (Nos. 1, 2 and 3), harbouring hæmolytic mastitis streptococci in two, two, and three quarters of the udder respectively, were kept at the Institute, and examinations of quarter samples were made three times weekly for a period of two months, and afterwards at intervals until they were dry. In these cows small clots were occasionally noticed in the fore milk, and the affected quarters were somewhat indurated. Streptococci were invariably detected by plating 1·0 c.c. of the fore milk, and only on very rare occasions were they absent when 0·1 c.c. was plated. The result showed that counts from 0·1 c.c. milk from the same quarter may vary from " innumerable " to less than 100 even at such short intervals as two or three days. Variations of this magnitude naturally have real significance.

Series III.—During 1929, three cows infected with non-hæmolytic mastitis streptococci were kept under observation.

Cow No. 4 was infected in the right fore quarter with non-hæmolytic streptococci (subgroup I). The teat was rather firm and the quarter slightly atrophied when compared with the others, and the milk secreted constantly contained clots.

Cow No. 5 was infected in the left hind quarter with non-hæmolytic streptococci (subgroup II). This quarter was distinctly smaller and firmer than the others, and was more sensitive when handled. It was only when the cow was nearly dry, however, that the milk was definitely clotted.

Cow No. 6 was also infected in the left hind quarter with non-hæmolytic streptococci (subgroup II). The quarter was almost completely atrophied, and showed subcutaneously on its posterior aspect an abscess from which streptococci of the same variety were recovered.

Quarter samples from cows Nos. 4, 5 and 6 were examined two or three times weekly for about three months, until the cows were dry. One c.c. of the secretion from the affected quarters showed on practically every occasion somewhat variable but always high streptococcus counts when plated in blood agar.

(*b*) Microscopic Examination of Milk Smears.

This method has been employed frequently for diagnosis, suitably stained smears of the deposit being examined for tissue cells on the one hand, or for diplococci or short or long streptococcus chains.

Examination for Streptococci.—It is no doubt true, as emphasised by Ernst (1909), that a positive diagnosis can be made with safety in cases where long streptococcus chains are present, composed of cocci arranged in palisade fashion, *i.e.*, chains in which the cocci are slightly elongated transversely. The presence of diplococci or short chains also indicates with fair certainty the existence of streptococcus mastitis. It is generally agreed, however, that a negative diagnosis cannot be made with safety when no chains can be detected.

From the results of Klimmer, Haupt, and Roots (1928) it may be seen that of 109 milk samples from which typical mastitis streptococci were grown, microscopic examination of smears gave negative results in 30 per cent., and unmistakably positive results in 35 per cent. only. Ehrlich and Bischoff (1930) found that, whereas it is possible to detect 94 per cent. streptococcus-infected cows by cultural means, only 41 per cent. can be detected by microscopic examination of sediment smears.

Our observations also showed the unreliability of the method in comparison with cultural examination, as may be seen from the instances given in Table 1.

TABLE I.

Streptococcus Colonies per 1·0 c.c. Milk	*Streptococci in Smears.*			*Total.*
	Numerous.	*Few.*	*None.*	
Over 8,000	39	29	17 (20)	85
1,000 to 8,000 ...	6	11	17 (50)	34
1,000 or less	8	24	72 (69)	104
	53	64	106 (47·5)	223

Note.—Figures in brackets represent percentages.

In these instances thin smears of the deposit were made and stained by the following method, which was found to be rapid and satisfactory :—

Methyl violet 6B	60 seconds.
Lugol's iodine	30 ,,
Acetone-alcohol (1 : 10)	10 ,,
Dilute carbol fuchsin	10 ,,

The results of the microscopic examination were recorded after a two-minute search, which is about the longest time that could be allowed in practice if many smears had to be examined. In order to rule out the personal factor, the results include observations by each of us.

Thus, of 223 instances in which streptococci were cultivated from 1·0 c.c. milk, these organisms were undetected in smears in 106, or 47·5 per cent. In cases where 1,000 colonies or less were grown from 1·0 c.c. milk, streptococci were not seen in smears in 69 per cent. Even when the number of colonies exceeded 8,000, streptococci were undetected in smears in 20 per cent. of cases. In these smears of milk direct from the udder, elongated cocci of the milk-souring type were not seen, so that no confusion arose from this cause.

Examination for Cells.—So far as tissue cells are concerned, an examination may be made conveniently of the cream which has risen after the milk samples have been standing for not less than two hours. Smears of medium thickness are fixed by heat and stained for a few minutes with 1·0 per cent. watery methylene blue and examined with a view to forming an opinion as to whether cells are present in excessive numbers. In one experiment* cream smear examinations were made of twenty quarters from which at the same time mastitis streptococci were cultivated, and of fifty-five quarters which were healthy in this respect. In the series of twenty infected quarters there were at least three, and possibly five, quarters in which the numbers of cells were only slightly increased. In the series of fifty-five quarters from which no streptococci could be cultivated, there were sixteen quarters in which the cell content was put down as slightly excessive, and at least seven, and possibly twelve, quarters in which the cell content was definitely excessive. In the latter case streptococci may have been present, but were not isolated, or the disturbance may have been due to other causes. Cases such as these are to be expected, and introduce difficulties ; apart from that the method is simple, and often permits a speedy decision as to the condition of the quarter.

* In this part of the investigation we derived benefit from the collaboration of Mr. A. D. Gill, M.R.C.V.S., of the Veterinary Laboratory, Wallaceville, New Zealand, who was on a visit to this country. Mr. Gill placed his experience of cytological diagnosis of cream smears at our disposal, and is responsible with us for the results of this experiment.

(*c*) Estimation of the Amount of Deposit Obtained by Centrifuging a Fixed Volume of Milk.

Trommsdorff (1906, 1909) introduced a simple method for the diagnosis of chronic streptococcus mastitis which aimed at estimating the leucocyte content of milk samples. 10·0 c.c. of a mixed sample of milk from the four quarters were centrifuged for a few minutes at about 1,200 revolutions per minute in a special tube, the lower part of which was narrowed into a capillary and graduated for special purposes at 0·001 c.c. intervals up to 0·02 c.c., and for practical use at the 0·01 and 0·02 c.c. marks. Samples from healthy udders gave deposits which were nearly always less than 0·01 c.c. (one part in 1,000). A deposit equalling 0·01 c.c. was regarded as suspicious, while if the amount of deposit reached 0·02 c.c. the result would almost certainly indicate infection of the gland. Many hundreds of tests showed that if the volume of the deposit from a mixed sample of milk from the four quarters was over 0·01 c.c. one or more quarters were excreting an excessive number of leucocytes, and this in the majority of instances depended upon a streptococcus infection of the gland.

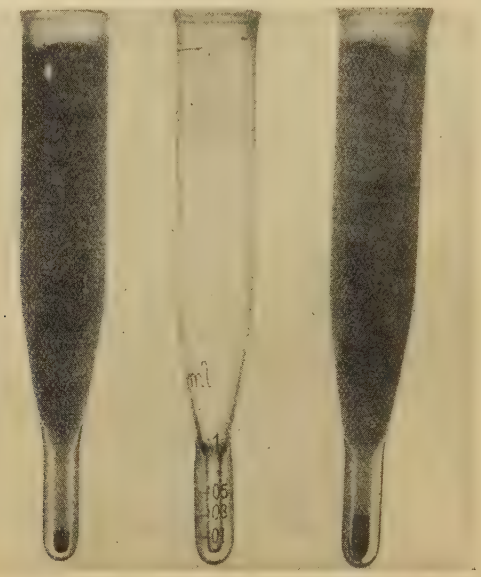
Unfortunately the tubes used are expensive, fragile, and difficult to clean, and it is not easy to get at the deposit with a platinum loop. As pointed out by Schuppius (1907), there may be considerable errors in graduation. With some tubes the error about the 0·02 c.c. mark varied from 25 to 40 per cent. on the minus side.

Ernst (1910) examined 276 samples of mid milk from streptococcus-infected quarters; of these it may be gathered from his tables that 113 (40 per cent.) showed deposits of less than 0·01 c.c., and 163 (60 per cent.) showed deposits of 0·01 c.c. and over, while of twenty-one similar samples from quarters which were considered to be free from streptococcus infection nine showed deposits of 0·01 c.c. or over. In forming an opinion as to the state of health of the quarters from which these twenty-one samples were derived, chief reliance appears to have been placed on repeated microscopic examination. Proscholdt (1928) examined 289 quarter milk samples, normal in appearance, or only slightly altered, but known to contain streptococci. The Trommsdorff test was positive in 203 or 69 per cent., *i.e.*, in 31 per cent. of the cases the volumes of sediment were considered normal. Rullmann and Trommsdorff (1906) found that with quarter samples a deposit higher than 0·01 c.c. was usually accompanied by a high streptococcus count on agar plates seeded with 0·05 c.c. milk. In many instances, however, such agreement was not noted, a high streptococcus count being co-existent with a low leucocyte figure. A high leucocyte deposit with absence of streptococci was noted less frequently. Ruhm (1908) and Ernst (1910) found that the absence of a high deposit does not necessarily indicate the absence of infection, while Trommsdorff himself (1909) pointed out that the test might be positive one day and negative on another. Sheather (1924), using the ordinary conical centrifuge tube, also concluded that the amount of sediment from 10·0 c.c. milk should be more than a " large speck " (about 0·03 c.c.) before it should be put down as excessive.

At the beginning of this work we made use of ordinary conical centrifuge tubes of 11 to 12 c.c. capacity, centrifuging 10·0 c.c. milk for five minutes at approximately 2,500 revolutions per minute (see later, page 177). Deposits were read as " trace," signifying the amount of deposit which could be regarded as normal; " more than slight," signifying the smallest amount which could reasonably be considered excessive; " slight," for intermediate or borderline

amounts. Volumes greater than " more than slight " were measured.

Such estimations were necessarily very rough, particularly since the lower extremity of the tubes varied greatly in size and shape. It was therefore obviously desirable to devise a modification which would permit more exact measurement of small amounts of deposit. Eventually a centrifuge tube* was adopted, similar in principle to



Special centrifuge tubes with deposits approximating 0 01 and 0·03 c.c., with graduated standard tube.

that of Trommsdorff (see Fig). It was made by soldering a piece of thick glass tubing of narrow bore into a wider piece so as to form a strong centrifuge tube of 11 to 12 c.c. capacity, which was then graduated at the 10 c.c. mark. The lower narrow part was ungraduated, 2 cm. long, and had a capacity of about 0·08 c.c. It was sufficiently narrow to permit readings as low as 0·005 c.c., and at the same time wide enough to allow the passage of a platinum loop. These tubes are inexpensive, strong, and easy to clean. In taking readings comparison may be made with a graduated standard in which volumes of 0·01, 0·03 and 0·05 c.c. are shown by means of variously-coloured layers of barium sulphate. With a little experience, however, the standard tube may be dispensed with.

It is evident that an opinion of what constitutes an excessive deposit must be governed by considering the amount of deposit obtained by centrifuging samples of milk from normal quarters. It is also evident that the only way by which it is likely that the state of a quarter may be judged is by means of a cultural examination of

* These centrifuge tubes, as well as the standard tubes, may be obtained from the Scientific Supplies Co., Ltd., 52 Hatton Garden, London, E.C.1.

This article was being prepared for the press when a publication dealing with the examination of milk appeared by Gressel and Schönberg (1930), in which is described a thick-walled centrifuge tube similar in shape to that now being used by us.

the milk from it. We have found that in quarters in which repeated cultural examination has failed to show streptococci or other bacteria which could be regarded as pathogenic, the amount of sediment obtained by centrifuging 10 c.c. of the fore milk was almost consistently less than 0·01 c.c., whereas in " infected " quarters milk samples may give deposits much higher than this.

Table II shows among other details the amounts of deposit obtained by centrifuging 10 c.c. quantities of milk from 485 samples from healthy quarters and from 326 samples from streptococcus-infected quarters. It is necessary to emphasise that the term " healthy " is used in the broad sense, and refers to freedom from both streptococci and other bacteria which there was any reason to suspect might be pathogenic. In most cases the samples were derived from different quarters ; if from the same quarter a period of several weeks usually intervened. The great majority of the samples were examined after overnight refrigeration (see later, page 177).

TABLE II.

AMOUNTS OF CENTRIFUGE DEPOSIT AND THE REACTIONS OF SAMPLES OF FORE MILK FROM HEALTHY QUARTERS AND FROM QUARTERS INFECTED WITH MASTITIS STREPTOCOCCI.

Healthy Quarters.			*Streptococcus-Infected Quarters.*		
Amount of Deposit.	*Reaction.*	*No. of Milk Samples*	*Amount of Deposit.*	*Reaction.*	*No. of Milk Samples.*
Less than 0·01 c.c.	Normal	434	Less than 0·01 c.c.	Normal	74
	Intermediate	24		Intermediate	40
	Abnormal (Alkaline)	5		Abnormal (Alkaline)	13
		463			127
0·01 c.c. or over	Normal	15	0·01 c.c. or over	Normal	43
	Intermediate	2		Intermediate	93
	Abnormal (Alkaline)	5		Abnormal (Alkaline)	63
		22			199
Total Number of Milk Samples, 485			Total Number of Milk Samples, 326		

The milk samples were from 210 healthy quarters and from 114 streptococcus-infected quarters. 10 c.c. amounts of milk centrifuged at 2,500 r.p.m. for five minutes. Reactions taken by means of brom cresol purple paper.

In the case of the healthy quarters it is seen that of 485 samples the deposit was less than 0·01 c.c.—usually well under this figure—

in 463 (95·4 per cent.), and 0·01 c.c. or over in twenty-two (4·5 per cent.), though it should be noted that in only three of the latter did the deposit exceed 0·015 c.c. With the infected quarters, of 326 samples, the deposit was 0·01 c.c. or greater in 199 (61 per cent.) and less than 0·01 c.c. in 127 (39 per cent.). No doubt if the examination had been repeated several times at intervals a larger percentage of infected quarters would have shown high deposits.

So far as the actual amount of deposit is concerned, this may vary in abnormal cases from 0·01 c.c. to 2·0 c.c., or even more, and with the smaller increases there may be no alteration in the appearance of the milk. Of the 199 samples from infected quarters in which the deposit was 0·01 c.c. or over, the amounts of deposit lay between 0·01 c.c. and 0·025 c.c. in 131 (65·8 per cent.), between 0·026 and 0·05 c.c. in 36 (18 per cent.), and over 0·05 c.c. in 32 (16 per cent.). According to these figures, in more than three-fifths of the samples from infected quarters the deposit does not rise much above 0·01 c.c.—an additional reason for keeping the limiting value low. Thus, we can only conclude that 0·01 c.c. deposit from 10·0 c.c. milk is not only suspiciously high, but can be regarded in most cases as indicative of pathological disturbance in quarters which are in full secretory activity.

We do not wish to imply that deposits less than 0·01 c.c. may not have pathological significance at times. Attention has been repeatedly directed to the differences in physical characters between the deposits from healthy and from infected quarters. With samples from healthy quarters, apart from a trace of " dirt " at the bottom of the tube, the small quantity of deposit is whitish, and often not very conspicuous, because it is not sharply marked off from the overlying milk. With pathological samples the deposit is distinctly yellowish and sharply marked off from the supernatant liquid, which in the narrow part of the tube is usually more or less transparent. In forming an opinion, therefore, as to whether a small deposit is normal or not, we have relied not only upon its amount, but also upon its appearance.

Exceptionally, we have found an increased amount of deposit to be the only indication of abnormality subsequently proved to be due to streptococci. Cases were also met with in which there was disturbance apparently not caused by streptococci, since these organisms could not be detected on repeated cultural examination. Such instances no doubt correspond to the " non-specific disturbances " referred to by Seelemann (1928), and in these, so far as can be judged, the increased cellular output would appear to be caused by the presence of other organisms, such as staphylococci.

(*d*) Estimation of the Reaction of the Milk.

Brief reference only will be made to this method, because it has been discussed fully by Stableforth (1930) in a recent paper from this Institute.

From Table II it may be seen that of 485 milk samples from

healthy quarters the reaction to brom cresol purple was normal in 449 (92·5 per cent.), and frankly alkaline in ten only (2 per cent.). Of 326 samples from streptococcus-infected quarters the reaction was definitely alkaline in 76 (23 per cent.), judged to be intermediate or slightly alkaline in 133 (40·7 per cent.), and normal in 117 (35·8 per cent.).

Influence of Certain Factors other than Disease upon (c) the amount of Centrifuge Deposit, or (d) the Reaction of Milk.

i. *Stage during the lactation period at which samples are taken.*—It is well-known that samples taken during or immediately after the colostral period show increased alkalinity and cell content. The same is true of cows which are nearing the end of their lactation period. Samples for examination should therefore be taken when cows are in full milk.

In order to find how long before the actual time of drying-off these changes may be expected to take place, quarter samples from two uninfected cows were examined weekly throughout the final three months of lactation. In both animals the fore milk of every quarter was excessively alkaline for about four weeks before drying, and this change occurred at least a week in advance of any notable rise in the cell content. During this final period the deposits commonly reached 0·03 to 0·08 c.c., and in two or three instances reached 0·1 to 0·2 c.c.

ii. *Stage during milking at which samples are taken* (see also page 168).—In streptococcus-infected quarters the amount of centrifuge deposit tends to be slightly higher with the fore or strip milk than with the mid milk. The reaction of fore milk often appears to be rather more alkaline than that of samples taken later in the milking, an effect which may be due to the slightly lower proportion of fat in the fore milk.

iii. *Speed of centrifuging.*—In our work, as a routine, milk samples have been centrifuged in an electric centrifuge for five minutes at 2,500 revolutions per minute. Centrifuging at lower speeds tends to reduce the amount of deposit, *e.g.*, when an ordinary hand centrifuge is used the amount is reduced by about one-sixth. This is not important in the case of larger deposits, but it is well to bear the tendency in mind when reading small deposits.

iv. *Fat content of the milk.*—Variations in the fat content within normal limits do not influence the amount of deposit. It is known that a high fat content, by masking the tint of the indicator, interferes with the reading of the reaction when colorimetric methods are used.

v. *Refrigeration of the milk sample.*—Following overnight cooling in the ice chest to 2° to 5° C., the deposits from the milk of infected quarters often showed a tendency to increase, the volume occasionally being nearly twice as great as before. In our work practically all samples were examined after refrigeration, so that the volumes

mentioned must be read in the light of this fact. It is of practical importance, however, to notice that deposits from uninfected quarters showed as a rule no appreciable increase after refrigeration, so that difficulty from this source is not likely to occur.

Relationship Between Reaction of the Milk and the Amount of Centrifuge Deposit.

Samples from quarters free from mastitis streptococci and which have a normal deposit usually have a normal reaction, and samples from streptococcus-infected quarters in which the amount of deposit is excessive show in many cases a slightly or decidedly abnormal reaction.

Information on these points may be deduced by reference to Table II.

Of 463 samples from healthy quarters in which the deposit was less than 0·01 c.c., the brom cresol purple reaction was that of normal milk in 434 (93·7 per cent.), and excessively alkaline in five only (1 per cent.). Of twenty-two samples in which the deposit was 0·01 c.c. or over, the reaction was excessively alkaline in five and normal in fifteen.

In the case of 127 samples from infected quarters in which the deposit was less than 0·01 c.c., the reaction was normal in 74 (58·2 per cent.), and frankly abnormal in 13 (10·2 per cent.). With 199 samples in which the deposit was 0·01 c.c. or over, the reaction was slightly or decidedly abnormal in 156 (78·3 per cent.), and normal in 43 (21·6 per cent.). In this series of 199 samples, however, 93 reactions (46·7 per cent.) were read as " intermediate " or " doubtful."

Not only was a direct relationship noted between the reaction and the amount of deposit, but it was also evident that when the amount of deposit was excessive the alkalinity of the sample increased in proportion to the volume of deposit present. Thus, with sixty-three samples in which the reaction was distinctly alkaline the amount of deposit averaged 0·097 c.c., with sixty-four samples in which the reaction was considered just abnormally alkaline the amount of deposit averaged 0·046 c.c., while with thirty-nine samples in which the reaction was read as intermediate the amount of deposit averaged 0·024 c.c.

The variations from week to week in the reaction and amount of deposit from the same infected quarter are illustrated in the Chart.

Relationship Between the Streptococcus Count of Milk, the Reaction, and the Amount of Centrifuge Deposit.

No close relationship could be observed between the actual numbers of streptococci present in the samples from infected quarters and the reaction of the samples or the amount of centrifuge deposit. This statement is based on an examination of milk samples from cows Nos. 1, 2 and 3, mentioned earlier as infected with hæmolytic

mastitis streptococci. In the case of 165 quarter samples from these cows the amount of deposit was measured, and in ninety-five of the same samples the reaction could be recorded as normal or distinctly alkaline. The results are shown in Table III.

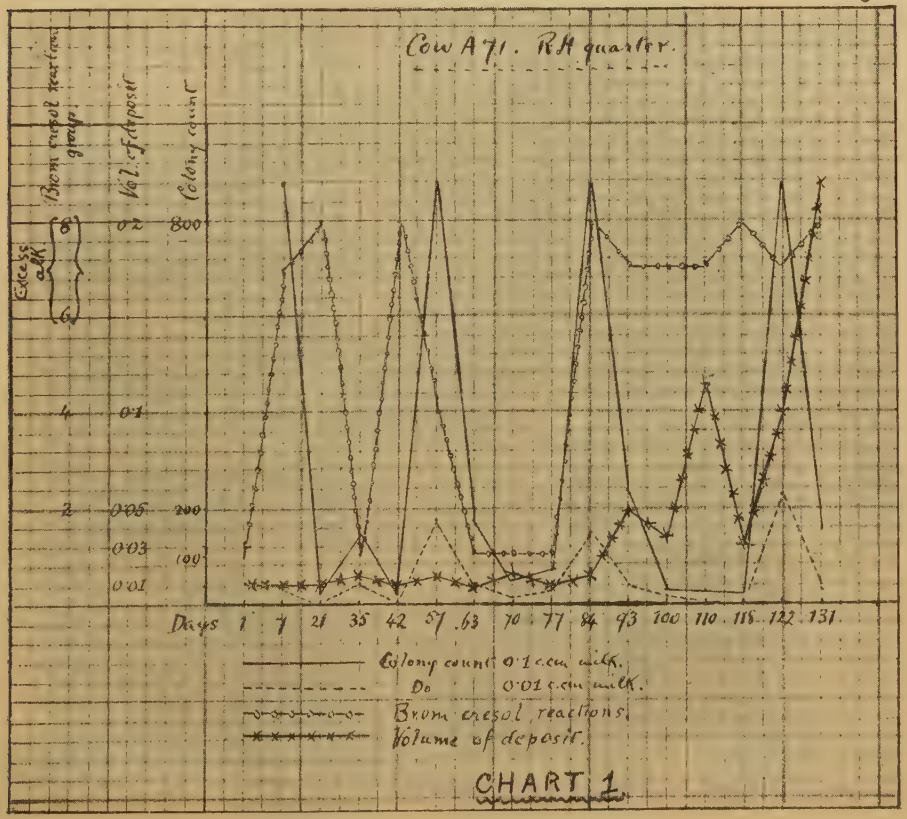
TABLE III.

Colony Count per c.c.	*No. of Samples.*	*Excessive Deposits.*	*No. of Samples.*	*Distinctly Alkaline.*
8,000 or over... ...	70	46 (65·7)	41	26 (63)
2,000 to 8,000 ...	47	33 (70)	22	12 (54)
Less than 2,000 ...	48	26 (54)	32	6 (19)
Total Number of Samples	165	105 (63)	95	44 (46)

Figures in brackets express percentages.

Diagnosis by Examination of Mixed Milk from the Four Quarters of the Udder.

It would be a great advantage if a mixed sample of milk from the four quarters could be used satisfactorily for diagnosis instead



of separate quarter samples, because in that case the labour of examination would be considerably reduced. Mastitis, however, is often confined to one or two quarters of the udder, so that to be reliable the method of diagnosis used must be capable of detecting abnormality in a milk sample which has been diluted three times with normal milk. Where the work has to be carried out in herds on a large scale the reliability of a method under these circumstances would become the essential test of its practical value. In order to obtain further information on this point, mixed samples of milk from infected cows have been submitted to cultural examination and to the sediment and reaction tests.

i. *Cultural examination.*—Observations were made on cows Nos. 1, 2 and 3, infected in two, two, and three quarters, with hæmolytic streptococci, and on cows Nos. 4, 5 and 6 each infected in one quarter with non-hæmolytic streptococci. The mixed samples were centrifuged, and of the deposit about one-half was plated direct in blood agar, and the remaining half diluted with nine volumes of saline, of which 1 c.c. was plated. At intervals over a period of three months, eighty-four mixed samples were collected from cows Nos. 1, 2 and 3, and in all cases but one streptococci were cultivated from the sediment, though in a few cases not more than ten colonies were present on the second plate. With cows Nos. 4, 5 and 6, mixed samples from each cow were examined at the same time as quarter samples on at least twenty occasions, and infection was demonstrated culturally in all cases.

Observations on mixed samples collected in the field from cows excreting hæmolytic streptococci confirmed these good results. Thus, forty-eight instances were noted in which it was possible to detect infection by means of a mixed sample, and in no case in this series was it not possible to show a positive finding. In ten of these instances the cow was infected in one quarter only. Conversely, when the mixed sample showed no mastitis streptococci these could not be detected in quarter samples.

ii. *The sediment test.* Trommsdorff (1906) concluded that even from a quantitative standpoint this test was of value for diagnosis when applied to a mixed milk sample from the four quarters, of which one quarter only was giving a high deposit. A number of figures in connection with this point are given by Rullmann and Trommsdorff (1906), but inspection of their figures shows that in many cases the volume of deposit from the mixed sample was considerably less than would have been anticipated from the amounts shown by the quarter samples, due to errors in the relative amounts of secretion taken from each quarter.

Naturally the amount of deposit obtained by centrifuging a mixed sample will depend chiefly upon the amount of sedimentable material in the samples from the infected quarters ; unless this reaches a certain level, the deposit from the mixed sample will not be excessive. The value of the method will be diminished for this reason, though it may still be possible to suspect infection from the appearance of the sediment. Our results also have shown that useful information may frequently be gained by inspecting the deposits from mixed

milk samples, but that in practice the amount of deposit from an infected quarter is sometimes not high enough to make the average abnormal.

iii. *The reaction test.*—This is intended for use with quarter samples only. Experiments have shown that the method is of small value when applied to mixed samples, since when samples from one or even two quarters were excessively alkaline the mixed sample may give a normal reading.

Discussion.

The present paper is concerned with the diagnosis of those forms of bovine mastitis which are not immediately apparent on manipulation of the gland or inspection of the milk. In nearly all such cases the cause of the disturbance is a long-chained streptococcus which in blood agar produces either hæmolytic or non-hæmolytic colonies. In this form the changes are essentially of a chronic catarrhal nature, with slowly progressive destruction of the milk-secreting tissue. In the less common but more acute forms of mastitis caused by certain types of non-hæmolytic streptococci, clinical examination alone is often sufficient to establish a diagnosis, because the attack starts acutely and often leads to rapid atrophy of the affected quarter. Nevertheless, even in this form the changes are sometimes more subacute, rendering laboratory assistance advisable. In general, clinical methods of diagnosis, such as palpation of the gland, are of limited value, even when supplemented by an inspection of the milk and by a knowledge of the animal's history. Owing to the varying size and consistence of the quarters of different cows, it is by no means always a simple matter to form an opinion as to the condition of the gland, so that even after a careful examination one may be left in doubt as to whether pathological changes are present. This is so particularly in the early stages of streptococcus mastitis ; in the later stages the gland is obviously enlarged and hardened. Laboratory methods of diagnosis may therefore be said to be essential, and the following are considered in this paper : cultural and microscopic examination of the milk, estimation of the amount of deposit obtained on centrifuging a fixed volume of milk, and estimation of its reaction.

1. In the *cultural work* blood agar poured plates have been chiefly used, and have been found to be of great value for the detection of hæmolytic streptococci even when only small numbers are present. They are also of great value when there are relatively large numbers of non-hæmolytic streptococci, but they are less useful when these organisms are present in only small numbers ; in this case colony picking for identification requires to be more exhaustive. As a general rule, however, the cultural examination of centrifuged milk sediment by blood agar plating, combined where necessary with the identification of questionable colonies, was reliable and satisfactory as a basis for comparison with the other methods investigated.

2. The *microscopic examination* of centrifuged milk sediment for

streptococci is much less reliable than the cultural method. In individual cases, or when small numbers of samples have to be examined, the method may give useful results, because if these are positive cultural work may be rendered unnecessary.

The microscopic examination of cream smears to obtain information as to the type and number of cells present is a simple and easily applied method which may serve to direct attention to abnormalities in the gland. As would be expected, however, there are a number of border-line cases in which a correct interpretation is difficult, and the method gives no definite clue to the species of bacteria responsible. On the other hand, in a small proportion of cases cytological examination may be the means of detecting disturbance which for some reason is not apparent by cultural methods at the time of examination.

3. Another comparatively simple method for detecting udder disturbance is to centrifuge a convenient volume of the milk, say 10 c.c., and to measure the *quantity of sediment*. This method was first brought into practical use by Trommsdorff (1906), though it was only intended as an indirect aid to diagnosis, and one which should be confirmed where possible by demonstrating the causal organism. The value of the test in practice has been upheld by some workers, but it has also been criticised on several grounds. Of these, technical objections to the form of tube recommended by Trommsdorff appear to be well founded. For routine work it is not practicable or necessary to use expensive graduated tubes, and in any case in bulk manufacture the accuracy of the graduations could not be relied upon. On the other hand, in our opinion something more efficient than the ordinary centrifuge tube should be aimed at ; for this reason we have used inexpensive and stoutly made tubes constructed in such a way that small volumes of sediment can be estimated easily and with accuracy sufficient for the purpose.

In a proportion of cases of streptococcus mastitis the centrifuge test fails as a means of diagnosis, because, as noted by earlier workers, at the time the test is made the milk contains no appreciable excess of sedimentable material. On the other hand, there are occasions when a slightly excessive deposit on centrifuging is the first indication of abnormal conditions which may be due to streptococci or to other organisms. In general, however, the result of the test is only significant when the amount of deposit is definitely excessive. This was recognised by Trommsdorff, who pointed out that the method only approaches infallibility when applied several times at short intervals. Apart from this, the opinion has been expressed (Ernst, 1910) that even with quarter samples no attempt should be made to set a limiting value between the deposits of infected quarters and of quarters considered to be uninfected, because of the variations which occur with the latter. Moreover, the deposit does not consist solely of cells, and therefore its volume does not necessarily correspond with the number of cells present. Finally, there is the objection that, when pathologically altered milk samples are centrifuged, a variable proportion of cells rise with the cream (Ernst, 1910 ; Skar, 1928).

An attempt to set a limiting value between sediments which are normal and those which are abnormal in amount obviously has to be made with care, not because the amounts vary greatly in milk samples from healthy quarters, but because it is by no means a simple matter to be sure that a quarter is really healthy. We believe that the discordant views of various workers as to what amount of sediment should be classed as normal are due to the varying procedures adopted for deciding whether a quarter is healthy. The procedures used do not appear to have been sufficiently searching in all cases, and the same critical remark may be applied to much of the work which has been carried out on the counting of cells in milk. Therefore, before deciding whether a quarter should be classed as healthy, we have made several examinations of the milk at intervals by cultural and other methods, the milk being drawn from quarters clinically healthy and with no history of abnormality, and from cows in regular milk and sound in their general health. Thus we would define a quantitatively normal sediment as that which can be obtained by centrifuging rapidly (2,500 revolutions per minute) a fixed volume of fore milk, say 10 c.c., for a constant time, say five minutes, the milk being taken from a clinically healthy quarter which several bacteriological examinations have shown to be free from pathogenic bacteria. Proceeding in this way, experience with a very large series of cases has shown that the amounts of sediment obtained by centrifuging 10 c.c. fore milk from " normal " quarters have been nearly always less than 0·01 c.c. (usually as low as 0·002 c.c.), even in quarters with a comparatively high count of ordinary udder bacteria. This at once suggests that quantities as low as 0·01 c.c. should be classed as abnormal, and colour is lent to this suggestion by the finding that with known streptococcus-infected quarters deposits are frequently little, if at all, higher than 0·01 c.c. Under strictly defined conditions, therefore, we have regarded 0·01 c.c. as representing that smallest volume of deposit which in practice should be classed as abnormal. It must be again emphasised, however, that deposits even smaller than this should be regarded as pathological if their physical characters are distinctly abnormal.

The fact that milk sediment contains other substances than body cells, such as fat, mucus, and red blood corpuscles, may be accepted. Schuppius (1907), for instance, points out that the sediment consists largely of fat, and may contain relatively few leucocytes or other cells. These objections, however, are not serious, since, as pointed out by Trommsdorff (1907) and by Ernst (1910), they do not destroy the practical usefulness of the test.

The objection that during the centrifuging of abnormal milk samples variable amounts of cellular material rise with the cream requires closer consideration, because it is obvious that the failures of the sediment test might be explained in this way. For the same reason, methods for determining the cell count of milk, involving the use of the centrifuge, have been condemned as unreliable (Prescott and Breed, 1910). It has been pointed out that the quantity of

cellular material which is carried upwards depends upon the fat content of the cells. If there is much fatty degeneration, a change which is specially liable to occur in chronic forms of mastitis, the specific gravity of the cells will be less than that of the suspending fluid and they will rise with the cream. Skar (1928) recommends that in suspected cases of mastitis in which the deposit is low the cells should be separated from the cream layer by shaking it up with a fluid of suitable specific gravity, such as 30 per cent. methylated spirit, and re-centrifuging. This is, of course, somewhat laborious, and could not be adopted as a routine where a large number of samples have to be dealt with. The important question in practice is to what extent the rising of cells with the cream interferes with the practical value of the sediment test. Examples given by Skar may be quoted in connection with this point. In a series of thirty-one abnormal milk samples in which the cells in the cream were between one and a half and twenty times as numerous as in the sediment, it is apparent that a positive diagnosis could still have been made in twenty-one instances if deposits of 0·01 c.c. and over had been regarded as excessive, and possibly in a still higher proportion if attention had also been paid to the characters of the deposit. If a knowledge of the total cell content is really necessary, the only alternative to separating the cells by centrifuging would be to carry out cell counts on whole milk, a procedure which would be still more laborious, would lack precision, and in any case would scarcely justify the expenditure of so much time.

4. The *reaction test* shares some of the drawbacks of the sediment test. Milk from infected quarters often shows a normal or at most an intermediate reaction not easy to interpret. The presence of fat is also an interfering factor. Nevertheless, when carried out by means of papers impregnated with the indicator the test is simple to perform, and the results are often suggestive or confirmatory of udder disturbance. There are even occasional cases in which an abnormal reaction, alone or in company with a slightly increased deposit, gives the first warning of infection that at the time is not revealed by cultural examination.

5. A laboratory procedure, to be suitable for large-scale herd diagnosis, must be reliable, reasonably simple to carry out, and as economical as possible from the point of view of personnel and materials. In view of the high incidence of occult mastitis infections in herds, it would probably be waste of effort, as well as demanding too much of the procedure, to apply the method in the first place to *mixed samples* from groups of five to ten cows, as has been suggested. It should, however, be reliable for detecting infection in a mixed sample of milk from the four quarters of a cow's udder of which one quarter only is infected. In these conditions cultural examination is of greater service than the sediment test, but, since it is necessary to centrifuge samples, an opportunity is afforded for observing the amount and characters of the deposit and, if desired, for making a microscopic examination. The information thus obtained may be

deemed sufficient, and cultural examination would then be superfluous.

Summary.

1. In this paper four methods which have been put forward for the diagnosis of occult forms of chronic streptococcus mastitis have been compared. Of these, cultural examination of centrifuged milk sediments by deep plating in blood agar was the most reliable. This medium is of great value for the detection of small numbers of hæmolytic streptococci and for the detection of non-hæmolytic streptococci when these are present in large numbers. When few non-hæmolytic streptococci are present blood agar is a less useful medium, owing to the difficulty of distinguishing the streptococcus colonies from those of bacteria normally existing in udder milk.

The samples to be examined should be taken from the fore milk rather than at a later stage in the milking, as the numbers of streptococci tend to be higher in the fore milk, and it is preferable to sow plates from the centrifuge deposit rather than from the cream. Storage of milk samples for a few hours at 18° C. sometimes causes a fall in the numbers of streptococci growing on plates, but this appears to be of little importance from the point of view of practical diagnosis.

During the course of chronic streptococcus mastitis there are often wide variations in the numbers of living streptococci in the milk, and at a given moment very few or no streptococci may be cultivable. Failure to cultivate streptococci is not unusual when the milk secretion is greatly altered in character.

2. The microscopic examination of centrifuged milk sediment for streptococci is far less reliable for diagnosis than cultivation in blood agar. In a series of 223 culturally positive cases a diagnosis could be made microscopically in only 52·5 per cent.

Cytological examination of smears of gravity cream is a simple and rapid method which has a certain indirect value for diagnosis, though a number of border-line cases are met with in which a correct interpretation is difficult.

3. Observation of the amount and characters of the deposit obtained by centrifuging a fixed volume of milk often gives valuable information as to the condition of the gland. The centrifuging may be carried out conveniently in the special tubes described.

Normally the amount of sediment from 10·0 c.c. milk is nearly always less than 0·01 c.c. (1 part per 1,000). This statement, however, should only be held to refer to the fore milk of cows in full and regular lactation, examined under constant conditions of speed and time of centrifuging, and in which repeated cultural examination fails to reveal mastitis streptococci.

With milk samples from streptococcus-infected quarters the sediment is often increased in amount, distinctly yellowish in colour, and clearly marked off from the supernatant fluid. The physical characters of the sediment may be distinctly altered without any

appreciable increase in the amount, *i.e.*, the volume remains below one part per 1,000. For practical purposes it is convenient to regard 0·01 c.c. altered deposit per 10·0 c.c. milk as the smallest amount which should be classed as abnormal. In more than half the number of streptococcus cases the amount does not reach 2·5 parts per 1,000. Under these circumstances the test may be said to give a positive result in about 70 per cent. of cases of streptococcus mastitis. As pointed out by Skar, some of the failures of the test may be explained by the fact that a proportion of the cells rise with the cream on centrifuging. In a few cases the sediment test gives the first warning of infection subsequently proved to be due to streptococci, and in a few other cases of disturbance apparently not due to these organisms.

Refrigeration of milk samples from infected quarters often causes a slight increase in the amount of centrifuge deposit, but does not influence appreciably the amount of deposit from normal milk.

4. Increased alkalinity of the fore milk of cows in full lactation is an indication of pathological disturbance within the gland. As with the sediment test, the result is only significant when the reaction of the milk is definitely abnormal. In about 35 per cent. of cases in which pathogenic bacteria, usually streptococci, can be demonstrated, there is no appreciable alteration in reaction ; in a further 40 per cent. the increase in alkalinity is only such as to raise a suspicion of infection.

5. A rise in the alkalinity of the milk is usually accompanied by, and is roughly proportional to, an increase in the amount of deposit on centrifuging. Neither of these changes bears any close relationship to the numbers of streptococci which can be cultivated from the milk.

6. Cultural examination of the centrifuged deposit is also of great value when applied to mixed samples of milk from the four quarters of an udder of which one quarter only is infected at the time of examination. The centrifuging of mixed samples at the same time affords an opportunity for inspecting the amount and characters of the sediment, from which valuable information may be derived. The reaction test with mixed milk samples is of practically no value for diagnosis.

The procedure outlined would be adopted in the first place for a preliminary sorting-out of infected cows and would be followed by an examination of quarter samples from the remaining animals. Such work could be readily undertaken by diagnostic laboratories on a large scale, without undue labour and with little special equipment.

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THE COMPARATIVE PATHOLOGY OF ANÆMIA AND LEUCOCYTHÆMIA IN FOWLS.

By H. P. Bayon.

Molteno Institute for Research in Parasitology, University of Cambridge.

Introduction.

"Anæmia" and leucocythæmia in fowls are diagnosed by examining the blood, but since birds suffering from any of these diseases present similar gross symptoms and the cellular elements found in the blood are not always distinguishable, because of pathological alterations, accurate classification can only be achieved by the subsequent microscopy of the inner organs and bone marrow.

These diagnostic examinations, combined with the observations of fowl-stocks during life, should reveal that pathological alterations of the cellular contents of the blood are quite common in fowls and that their causes appear to differ considerably. Not all these morbid conditions of the blood can be correctly termed "blood-diseases" or hæmopathies, indeed the term is usually a misnomer. In the following paper the expression "hæmopathy" will be used to denote a disease which begins, develops, and ends within the boundaries of the blood-forming or hæmopoietic tissues.

The hæmopathies of fowls have been previously observed by numerous authors, foremost among them Ellermann (1908 to 1921), and I have reviewed the literature in "Parasitology" (1929). Since then my attention has been called to two papers dealing with the subject. The first, by Csontos (1922), describes an outbreak of simple anæmia among fowls in Hungary; it was thought due to poor feeding. The anæmia does not appear to have been severe, because the erythrocyte count gave an average of 2,742,000 with 64,000 white corpuscles. The surviving fowls were cured by being fed with corn.

The other is a monograph by Battaglia and Leinati (1929), who after examination of several fowls with different hæmopathies and as a result of performed transmission experiments, conclude that anæmic hæmopathies originate in the marrow, but that the variations in the appearances of these diseases are due to differing reactive capacities of the fowls spontaneously affected or inoculated.

It is the general opinion that these hæmopathies correspond approximately to the similar diseases described in man and domesticated animals, but the main difficulty throughout has been to prove their pathogenetic origin and causation and correlate it with anything observable in mammals.

Several other complexities have obscured an understanding and delimitation of these ailments, but these will be discussed after a description of the morbid lesions of the principal types of hæmopathies met in fowls, and their consideration from the standpoint of comparative pathology; that is, to deduce from them information

which may help to elucidate the analogous diseases in man and animals and possibly guide the therapy for their alleviation.

Avian Hæmatology.

These avian morbid conditions originate as a selective disturbance of the process of hæmacytopoiesis, which includes the various stages in the development of the constituents of the blood. According to the lucid definition of Piney (1930), we have to regard the blood as a composite fluid containing the products of all the hæmatopoietic tissues. A brief recapitulation of recent advances in the knowledge of this subject will explain the genesis and manifestations of avian oligocythæmias and leucocythæmias.

In birds, erythrocytes take their origin from the lining endothelium of sinusoid vessels in the marrow, the principal deposit of which is found in the femur. The inner surface of this lining is seen to proliferate, gradually forming clusters of closely packed cells, whose cytoplasm is eventually loaded with hæmoglobin as they advance from the periphery to the centre. Once sufficiently mature, single erythrocytes appear to be loosened by flowing plasma and swept out into the general circulation.

Leucocytes, that is granulocytes, are formed by the proliferation of the surface of separate sinusoids, in a somewhat similar manner, except that intravascular cluster formation does not develop even when the leucocytes are closely packed, for the leucocytes congregate in groups outside the sinusoid vessels forming erythrocytes. Once matured, leucocytes appear to join the circulation by active motility. This dualism is possibly not always noticeable in normal bone-marrows, but is very evident in specimens from the morbid conditions we are considering.

Lymphocytes are produced in the marrow, where deposits can be frequently detected, but additional centres of activity are found in the lymphatic nodules scattered in the glandular organs and muscles of fowls.

Monocytes are of reticulo-endothelial origin and accordingly, though they can be found in the marrow, must also originate in other parts of the fowl's system, particularly the spleen and liver.

The genesis of blood-platelets or thrombocytes in birds is not easily determined, because the oval nucleated bodies, though smaller, resemble pale erythrocytes, but it is to be assumed that they are produced by the fragmentation of large cells of the megacaryocytic type.

Healthy hens average about three millions erythrocytes per c.mm. ; cocks show about 10 to 15% more. The hæmoglobin content is fairly regular at 60 degrees on the Sahli scale.

There is no conclusive agreement about the normal number of leucocytes and lymphocytes in fowls or their exact morphological differentiation. This is partly due to the rapid temporary leucocytosis which may arise in fowls owing to numerous causes.

Since, however, some standard must serve as a basis of any

pathological study, for the purpose of this paper the following figures have been considered to be the rule in healthy chickens :

Total white corpuscle number per cubic millimeter = 20,000.
Lymphocytes = 8–12,000. Granulocytes = 6–8,000.
Monocytes = 2,500. Uncertain = 5–700.

From the functional standpoint, it appears to be admitted that, concurrently with the normal fairly active production of erythrocytes in the sinusoids of the bone marrow, a regular and regulated destruction of erythrocytes takes place in the liver, spleen, and possibly also in the lymph nodes. This destruction is very much intensified in various pathological conditions, and appears to be connected with the production of some of the bile pigments. It is not ascertained that the liver possesses a hæmatocytopoietic function in the normal adult fowl, though this is evident in the embryo-chick.

I.—The Classification of Avian Hæmopathies.

Three main types of hæmopathies occur in fowls, with the following distinctive features :

I.—*Oligoerythrocytosis*, corresponding to "anaemia," but the former name indicates, more accurately, that the symptoms consist in the diminution of the number of red blood corpuscles and not really in a lack of blood. Should it be desired to be particularly accurate in diagnosis, one might distinguish : (a) *Oligochromhæmia*, where the red blood corpuscles are impoverished in hæmoglobin. (b) *Oligocythæmia* where the cells are reduced in numbers. Anæmia, properly speaking, occurs only after heavy losses of blood, when the total volume is acutely diminished.

To oligoerythrocytosis one may add : *Polyerythrocytosis*, a morbid and lasting increase of the numbers of red blood corpuscles, together with certain definite lesions of the marrow, liver, and spleen ; similar or analogous to Vaquez's disease in man. This, in fowls, is a condition allied to erythromyelosis which does not appear to have been previously described.

II.—*Leucocythæmia*, a pathological, persistent, and progressive increase in the circulation of the stem cells of the mature granulocytes usually present in healthy fowls. This is frequently accompanied by a concurrent oligoerythrocytosis.

III.—*Lymphocythæmia*, a similar pathological increase in the numbers of cells of the lymphocyte series, in which one may include monocytes. Here again oligoerythrocytosis is noticeable, particularly in the later stages.

II. Oligoerythrocytosis.

The oligocythaemic conditions which arise through the intensive parasitic infestation of chickens are well known and have been carefully studied in the particular instance of the nematode *Ascaridia lineata* (Schneider) by Ackert and Herrick (1928). These authors

found that the chicks which survived three weeks of parasitism usually recovered completely, gradually gaining in strength and improving in plumage until they eventually approached and sometimes even exceeded the controls in vigour, appearance, and performance.

Where oligocythaemia develops in these and similar instances it is the evident direct result of parasitism, and once the parasites are expelled or removed the hæmopathy is soon healed. Similarly, the oligocythæmia due to the depredations of the red mite, *Dermanyssus gallinae*, is a direct outcome of the persistent losses of blood, and once the depredations of the mite cease, the anæmia makes no further progress and is cured.

These anæmic symptoms are temporary in nature and the evident result of parasitism, but, apart from such symptomatic conditions, hæmopathies are observed in fowls which, though possibly originating through the action of some obvious helminth, yet progress independently of the initial infestation and lead on to the death of the fowl with the appearances of which is called "aplastic anæmia." Similar conditions form a class which ought to be separated from other oligocythæmias which are purely symptomatic, and to make the distinction quite clear I have suggested for these ailments the name "erythromyelosis," as diagnostic of their cardinal pathological feature.

Originally large numbers of such fowls were obtained from a poultry farm in Kent, and in this particular instance it was found that the fowls harboured in the duodenum large numbers of a minute cestode, *Davainea proglottina*, whose presence in numerous other fowls did not appear to be followed by definite hæmopathic symptoms. A full description of this outbreak is to be found in Vol. XXI "Parasitology" 1929, but the observations at necropsy are now tabulated for the first time (p.193). Similarly diseased fowls have been obtained from Wiltshire and elsewhere and in these no cestodes were found; thus clinching the suggestion advanced in my previous paper that the *Davaineæ* were an introductory but not essential cause of the hæmopathy.

(*a*) *Progressive Oligoerythrocytosis in Fowls—Erythromyelosis.*

This disease has been more frequently observed in light breeds, such as Leghorns, but other fowls are not immune. In large poultry-farms, with a thousand or more fowls, several birds may develop the ailment at the same time, with a predominance during the egg-laying periods, though one of the symptoms is delay or cessation of egg-laying. The comb and gills are very pale and may take on a lemon-yellow tinge. [For coloured photograph of condition see Bayon (1928).] The hens become quieter, are dull and weak, and if they are obliged to run about it is noticed that they easily get short of breath. There is no particular loss of appetite, nevertheless the birds do not thrive and their muscles feel flabby. Temperature is slightly below normal (105°—107° F.), as distinct

from the oligoerythrocytosis resulting from infections, such as chronic spirochætosis or tubercle. So far only hens have been observed with these symptoms, but the opportunity for the observation of large numbers of cockerels is necessarily limited.

1. *Symptoms and Pathological Signs.*

The blood is pale and watery, and it clots with difficulty. Erythrocytes number a million or even less for long periods of time, extending over several weeks or even months, with but relatively slight fluctuations. In this much reduced total, on a dry Leishman-stained slide, what one may call " normal " erythrocytes scarcely number a third. The rest show all degrees of immaturity, beginning from a round cell with a pale blue cytoplasm with a sharply defined round, mottled or " cartwheel " stained nucleus, and progressing to an erythrocyte slightly less oval than usual and whose cytoplasm has taken on a pale pink tinge. Granulocytes and lymphocytes are usually neither altered nor increased in numbers, but immature cells belonging to both series are to be seen. Cells of the lymphocytic group are distinguished from erythrogonial cells by the nucleus being usually smaller, more compact, and showing a slight indentation ; the cytoplasm is scanty, varies in breadth, and contains a few azurophile granules.

All stages in extrusion of the nucleus can be detected, ending in a ragged or distorted cytoplasmic remnant ; in acute or intense erythromyelosis two or even three unseparated erythrogonial cells may be found in the circulation ; they should not be confused with grouped thrombocytes, whose numbers and appearance seem but slightly altered.

The hæmoglobin content is somewhere in the neighbourhood of 15—20% on the Sahli scale. Hb-index = under 1.

On section the muscles are found pale and flabby or even wax-like. Liver often swollen, enlarged throughout, usually mahogany coloured or less frequently a deep yellow, without nodules or cheesy deposits ; section exudes a clear, pale pink serum, the liver substance is spongy and soft ; colour same as surface ; no nodules. Weight varies considerably according to the different stages of the disease (graduating between 100 grammes and 25), as the initial swelling may be followed by a terminal cirrhosis. Gall-bladder nearly always distended and full of dark bile. Spleen pale, frequently enlarged and swollen, but without the irregular nodulation which is a feature of tubercle ; section pale, uniform in colour. Weight usually two or three times increased, i.e., 2 or 3 grammes. The femur is nearly always thickened, the bone hard, in a condition which may be described as eburnation, though in certain acute cases the bone may be thin and eroded. In the former instance the scanty marrow is usually pale and watery, in the latter the marrow is deep red and succulent and frequently shows a hyaline currant-jelly-like appearance.

Microscopically, the lesions of the bone-marrow are an essential

NECROPSIES OF FOWLS WITH SPONTANEOUS ERYTHROMYELOSIS.

No.	Died or Killed.	1. General Condition. 2. Colour of Comb and Gills.	Liver Weight. In gms.	Spleen Weight. In gms.	Thigh-bone.	Marrow.	Davainea.	Remarks.
1	June 28th, 1927 ...	Emaciated ; pale yellow ...	—	—	—	—	—	—
2	July 12th, 1927 ...	Emaciated ; yellow ...	—	—	—	—	—	—
3	July 19th, 1927 ...	Oedematous ; pale yellow	—	2	Hard, thick...	Scanty...	—	—
4	July 19th, 1927 ...	Not emaciated ; pale ...	—	—	—	—	—	—
5	Aug. 22nd, 1927 ...	Not emaciated ; pale ...	—	7	Hard, thick...	Dark ...	—	—
6	Sept. 2nd, 1927 ...	Oedematous ; pale ...	—	—	Hard, thick...	—	—	—
7	Sept. 2nd, 1927 ...	Not emaciated ; flabby ...	54	4·2	Hard ...	—	++	—
8	Oct. 7th, 1927 ...	Emaciated ; pale pink ...	35	1	Not hard ...	Dark red	+	Chondro-fibroma.
9	Oct. 10th, 1927 ...	Emaciated ; pale pink ...	41	0·5	Hard, thick...	—	++	—
10	Oct. 29th, 1927 ...	Not emaciated ; pale yellow	31·5	3·5	Hard, brittle	Red ...	++	—
11	Nov. 15th, 1927 ...	Not thin ; pale	60	2	Soft ...	Red ...	?	—
12	Nov. 26th, 1927 ...	Not emaciated ; yellow ...	35	1	Not thickened	Dark red	None found	Peritonitis.
13	Dec. 6th, 1927 ...	Undersized ; yellow ...	44	0·8	Hard, brittle	Pink ...	++	—
14	Dec. 6th, 1927 ...	Not emaciated ; deep yellow	30	1·0	Hard, white	Light red	++	—
15	Dec. 21st, 1927 ...	Undersized ; pale yellow ...	29·5	0·8	Hard, thick...	Watery	++	—
16	Dec. 27th, 1927 ...	Emaciated ; very yellow ...	27	0·6	Hard, thick...	Dark red	+++	—
17	Dec. 29th, 1927 ...	Undersized ; very yellow...	15	0·6	Hard, thick...	Gelatinous	+	—
18	Dec. 31st, 1927 ...	Flabby ; deep yellow ...	67	1·7	—	—	None found	Not fresh.
19	Jan. 23rd, 1928 ...	Emaciated ; pale yellow ...	38	0·3	Thickened ...	Pink ...	++	Chondroma.
20	Jan. 25th, 1928 ...	Emaciated ; yellow ...	37	1·2	Thickened ...	Pink ...	++	—
21	Mar. 10th, 1928 ...	Emaciated ; undersized, very yellow.	54	0·7	Hard, thick...	Pale jelly	++	—
22	Mar. 12th, 1928 ...	Undersized ; pale	62	5·2	Very hard ...	Pink ...	++	—
23	Apr. 14th, 1928 ...	Not emaciated ; very yellow	60	1·2	Compact ...	—	—	—
24	Apr. 23rd, 1928 ...	Not emaciated ; pink ...	104	3·8	Nearly solid	Pale, scanty.	+	Liver treatment.
25	May 14th, 1928 ...	Not emaciated ; red with yellow tinge.	29	3·5	Not thickened	Dark red	++	—
26	June 10th, 1928 ...	Very emaciated ; congested	30	0·3	Hard ...	Dark red	+	—
27	July 9th, 1928 ...	Emaciated ; yellow ...	—	—	Thick ...	Pale ...	—	Not fresh.
28	July 20th, 1928 ...	Emaciated ; pale	30	0·2	Thickened ...	Red ...	+	—
29	July 4th, 1928 ...	Not emaciated ; pale yellow	Small	Small	Very hard ...	Pale ...	—	—
30	July 20th, 1928 ...	Emaciated	25	0·5	Hard, thick...	Pink ...	++	—

feature and consist of intensified intravascular production of erythrocytes in the sinusoids, noticeable through the presence of numerous adherent clusters. This activity may be followed, in the later stages, by exhaustion of proliferative power of the erythrogenic tissues and their substitution by fat and hyaline substances. In addition there is a much increased production of osteoid tissue.

The lesions in liver and spleen can be brought into line with the interpretation that the eruption of immature erythrocytes into the circulation, is followed by an enhanced destruction of these non-viable cells in the endothelia of the liver and spleen. Accordingly, there is a considerable dilatation of the inter-acinar capillaries in the liver ; a corresponding predominance of endothelial elements in the spleen, whose vessels are considerably thickened and histological features altered by a scattered proliferation of lymphoid cells, so that a distinction between Malpighian bodies and pulp is no longer possible.

Both in the liver and spleen irregular deposits of dark pigment are frequent, resulting from the destruction of erythrocytes.

2. *Causation.*

It can now be stated that this disease is due to one of those invisible microbes which go by the name of " viruses " and for which I have suggested the name " microplasm."

Since my last publication (1929) I have succeeded in transmitting the disease to further hens and, though I am fully alive to the possibility of these being birds which developed the disease spontaneously and not as a result of the injections, yet the necessary controls have been made. The presence of helminthic intestinal parasites is a contributory cause of erythromyelosis.

3. *Prevention and Treatment.*

Erythromyelosis is not " catching " or infectious in the commonly accepted meaning of the words, and therefore a strict isolation of the hens affected will not stamp out the disease. Even in massed outbreaks it is restricted to certain breeds or even strains in these breeds, and therefore any preventive measure should include a change of stock. Parasitic infestations should be dealt with ; in one particular instance the frequent incidence of erythromyelosis stopped when the fowls were changed from one ground to another, very probably owing to the cessation of the possibility of re-infection with *Davainea proglottina.*

As to treatment of valuable stock, I have found chopped raw liver beneficial; in measured quantities, however, since some fowls may devour a harmful excess. A small teaspoonful every other day is quite sufficient.

In my opinion the liver diet acts by inhibiting the hepatic destruction of erythrocytes.

4. *The Experimental Production of Poly-Erythrocytosis.*

It has been seen in the previous notes that in fowls the pathogenesis of one definite type of oligoerythrocytosis has been shifted from the liver or circulation to the marrow, where it is considered it takes its origin.

Numerous experiments were performed with the intention of transmitting the disease, apparently with no result except that after repeated injections with erythromyelosis blood transitory oligoerythrocythæmia followed, similar to the results obtained by Bedson and Knight (1924). After prolonged observation it became evident that in fowls of other breeds the injection of erythromyelosis blood resulted in an irritative condition of the marrow, with an excess of mature erythrocytes in the circulation, increase of hæmoglobin and on death a considerable enlargement of the liver and spleen, and hyperplasia of the red bone marrow, with cluster-formation of erythrocytes. This result was obtained in one batch of ten Leghorns and in single Rhode Island Reds inoculated.

Apart from the peculiarity that, following inoculation with oligoerythrocytosis, poly-erythrocytosis developed, the results are remarkable in that they support my interpretation of the lesions found in the fowls with spontaneous erythromyelosis.

It is seen that both diseases originate in the marrow and that the full clinical picture follows through the greater or lesser erythrophagia of the liver and spleen.

III.—Leucocythæmia.

This disease was first recognised in man by David Craigie (1845), whose clinical observations related to one case; this was followed by the description of a further case which was autopsied by J. H. Bennett (1845), in Edinburgh.

The latter author suggested that the disease was propagated by a process of " zymosis," that is, might be due to an infective agent. Craigie and Bennett considered that the outstanding pathological feature was the presence of pus-corpuscles in the blood-stream, in addition to the enlarged spleen.

Previous observers of similar conditions, like Bichat and Velpeau, did not differentiate such diseases from pyæmia, but Bennett, in 1852, proposed the name " Leucocythæmia " for the condition he had observed ; a name which was a good and accurate clinical designation of the disease, which then in course of time got definitely distinguished from several similar morbid conditions of the human blood-forming tissues.

Analogous alterations of the blood in different animals were subsequently recognized, some of them taking on an epidemic character and possibly not being strictly comparable to the genuine leucocythæmia known in man ; but though leucocythæmic appearances in fowls were previously mentioned (Butterfield 1905, Kon 1907), the first conclusive account of this particular disease as seen

in poultry appears to be that of Ellermann and Bang (1908), in Denmark. Prof. Vilhelm Ellermann continued these experimental observations during many years and proved that the disease could be transferred from one fowl to another by Berkefeld filtrates of the blood and organ emulsions.

As a result it has been established that the disease has a pathology of its own and that the type Ellermann calls " myelotic leucosis " behaves like certain tumours by disseminating secondary or metastatic deposits.

By some observers it has been suggested that there is a gradual clinical gradation between anæmia and leucocythæmia, and this is expressed by postulating the existence of an " erythro-leukæmic chain." I hold, however, that progressive anæmia on the one hand and leucocythæmia on the other are distinct diseases, and that, though severe anæmic symptoms occur in most instances of leucocythæmia, this anæmia is a secondary complication of the leucocythæmia.

A consideration of leucocythæmia in fowls should confirm this view.

(*b*). *Leucocythæmia in Fowls.*

The fowls suffering from this ailment do not differ much in appearance from those with erythromyelosis, particularly as oligoerythrocytosis is a fairly constant feature of the disease, once it is fully developed. Accordingly there is the same paleness of the comb and gills, though the yellow tinge which is so noticeable in erythromyelosis has never been found as intense in the fowls I have been able to observe. The hens stop laying, go light, are relatively quiet, in fact the distinction from erythromyelosis is only possible after careful microscopical examination of the blood.

It should not be forgotten that the symptoms I have described may easily occur as the result of tuberculosis, especially if there has been a localisation of the tubercles in the marrow and spleen, as is often the case. In such instances the simple examination of the blood may lead astray, as the leucocytes may be considerably increased and a conclusive diagnosis be only obtained after a differential count of the white blood cells and confirmation by a bacterioscopic examination for acid-fast bacteria.

One distinction which can be made in the incidence of the disease is that erythromyelosis is mostly a disease of young chickens, scarcely a year old, whilst leucocythæmia has been observed mostly in older hens.

1. *Pathology.*

The blood is watery and pale. Cellular content will number about one and a half millions to two millions, and on microscopical analysis it will be found that these may consist of one million of distorted and pale erythrocytes and 300,000 or more white blood cells, recognisable by a lobate nucleus, a pale protoplasm with

scanty badly staining granules; some of these may be detected actively dividing mitotically, whilst others are undergoing simple division. Mature granulocytes are not more numerous than usual in health. The eruption of pathological leucocytes into the blood-stream has taken place at an early stage, before the production of granula, though eosinophilic myelocytes may be found abundantly in the marrow. Hæmoglobin 20° or less; index usually slightly below 1.

Liver will be found enlarged to double its size, weighing 70-80 grammes or more: colour pale or mottled with smooth, white, round spots, which are also noticeable in the section of the liver substance. Here again a resemblance to tubercle is present, though tubercles are usually drier, on section have a cheesy or sawdust like appearance, are irregularly scattered and vary greatly in size, and produce a distorted enlargement of the organ affected. In all doubtful instances a bacterioscopic examination must decide. Spleen is also enlarged and presents the same pale, smooth appearance and regular whitish spots; it weighs 5 to 15 g. or more. The bones are not appreciably thickened, the marrow is pale or pink and diffluent. Microscopically, the numerous deposits in the liver and spleen are found to consist of round cells with a sharply defined nucleus and narrow cytoplasm, but also cells with lobate nuclei; mitotic figures are seen in large pale cells.

It is frequently noted that the hepatic cells are reduced to star-shaped groups, surrounded by compact masses of cells of leucocytic origin, so that these predominate in the microscopic field. No such substitution of liver tissue by extraneous cellular elements takes place in erythromyelosis; this is the most striking of many distinguishing features.

In the marrow there is a decided increase of the extra-sinusoidal cell-layers in definite centres of activity, and at the same time the usual intra-vascular clusters of erythrocytes may be observed at intervals among masses of myelocytic cells, but separated from them by a layer of undifferentiated endothelium.

2. *Causation.*

This is obscure, because though in certain fowls the cestode *Davainea proglottina* was found in great numbers in the duodenum, in others it was not present. The disease originates as a selective proliferation of the leucocyte-producing tissues in the marrow and a resulting disturbance of the normal process of entry of these cells into the circulation. This, in conjunction with the experiments of Ellermann (1921), seem to indicate that this form of leucocythæmia in the fowl is due to a "microplasm."

3. *Prevention and Treatment.*

The disease occurs predominantly in certain strains, so that the introduction of fresh stock may be recommended. The feeding with liver does not affect these cases, but it is thought that feeding with fresh vegetables influences the condition.

IV. Lymphocytic Diseases; Lymphocythæmia, Diffuse Lymphomatosis, Etc.

In man this group of diseases is clinically and pathologically quite distinct from oligoerthrocytosis on the one hand and leucocythæmia on the other, though both anæmic symptoms and an increase of leucocytes may complicate the blood picture seen under the microscope. Thomas Hodgkin (1833) first recognised one form of the disease in man, but in fowls lymphocythæmia was originally described by Warthin in 1908, though its sharp demarcation from the other diseases was not stressed.

It has become increasingly evident in course of time that there is a large group of morbid conditions in which an increase of lymphocytic elements cells takes place, either in the blood or lymph-glands and other tissues. Single instances of such diseases are often difficult to classify, even after clinical observation and anatomical examination; this is due to the fact that several cells exist in pathological conditions which can only be distinguished from lymphocytes after the most careful examination. Nevertheless, certain differences can be made out in the several lymphocytic diseases of fowls, because outbreaks of any disease of the group in birds of the same stock present on the whole fairly uniform characters.

(*c*) *Lymphocythæmia and Lymphomatosis in Fowls.*

Lymphocythæmia in fowls is usually classed together with a somewhat similar morbid condition, diffuse lymphoma, but the main distinction is to be found in the blood. A continued and progressive increase in the number of lymphocytes indicates that the morbid process is affecting the matrix of these cells in the marrow and elsewhere and inducing their premature appearance in the blood-stream in numbers far above normal.

In diffuse lymphoma in chickens the changes in the blood are by no means so intense, amounting to perhaps 100 000 lymphocytes per cmm., against the 2-300 000 found in lymphocythæmia; not only this, but in diffuse lymphoma the increase is subject to great fluctuations. This difference is due to the fact that here we are dealing with a simple multiplication and dissemination of lymphoid elements, apart from the mechanism of entry into the circulation which possibly can take place in the blood marrow and there only. The presence of lymphocytic deposits in the skin and inner organs may occur in any of the lymphocytic diseases.

1. *Pathology of Lymphocythæmia.*

Accordingly, in lymphocythæmia the hens present the same symptoms of paleness previously described, and, on examination, the blood is found to be thin and watery; a cell count will give the usual low figure of two millions and less, which, on microscopical examination will be found to consist of possibly one and a half millions of erythrocytes and the rest immature cells of the lymphocyte series. These present in certain stages a strong resemblance

to erythroblasts, but attentive examination will reveal differences in the majority of both varieties.

The inner organs, particularly liver, spleen, and kidney, are enlarged and studded with whitish cellular deposits, which on microscopical examination are seen to be predominantly cells of the lymphatic group ; mitosis will be observed in some.

The bones are not thickened as a rule, and the marrow is pale and reveals microscopically numerous areas of intense lymphocytogenetic activity in the spaces between the sinusoids, which show the usual clusters of erythrocytes.

2. *Characteristic Features of Lymphomatosis.*

In diffuse lymphoma the blood is not appreciably altered as it flows from a superficial wound, being red and clotting fairly easily. A typical cell-count would give the following figures : Erythrocytes 2 800 000, all white blood-corpuscles 60 000. Hæmoglobin 55%. The presence of scattered lumps between muscle and skin is easily detected and indeed generally calls attention to the ailment.

On the necropsy the appearance of the inner organs is similar to that described in the other diseases, that is, considerable enlargement of liver and spleen, due to lymphocytic deposits. The marrow, however, is not particularly affected and in all appearances differs but slightly from that found in normal birds.

A peculiar variety of this ailment causes fowl-paralysis ; this form is distinguished by an irregular accumulation of lymphocytic cells around nerve tracts.

3. *Pathogenesis of Lymphocytic Diseases in Fowls.*

In the foregoing remarks an attempt has been made to lay stress on what appear to be palpable differences between two types of spontaneous lymphocytic disease in fowls ; it is admitted that experimental work induces the belief that the ultimate causes of these diseases are closely allied, although diverse clinical pictures may develop in transmission by inoculation.

This was found to be the case with a fowl-disease which does belong to the group, and which Pappenheimer, Dunn, and Cone (1929) have named " neurolymphomatosis gallinarum," since the lymphocytic deposits congregate particularly around nerves, though such accumulations are also to be detected in other organs. One of Pappenheimer's pictures of lesions in the liver might well have been obtained from a typical case of lymphocythæmia. Pappenheimer, Dunn, and Seidlin (1929) succeeded in transferring the condition to 24 fowls out of 89 inoculated.

This avian ailment has been described by Galloway (1929) and MacGaughey and Downie (1930) in the United Kingdom, and they also comment on the irregularity of the lesions in the spontaneous cases observed. Notwithstanding a widespread distribution of lymphoid deposits throughout the body, no striking alteration of

the number of lymphocytes appears to have occurred in the blood and the marrow was unaffected.

These findings have confirmed the view I have held for some time, that lymphocythæmias originate in the marrow and that scattered proliferations of a lymphoid character develop from tissues in other parts of the system.

4. *Circumscribed Proliferations of Hæmatogenetic Tissues in Fowls.*

These proliferations are frequently grouped with hæmopathies, though the distinction is to be found in their being, as a rule, purely local; they do not scatter or metastasize like tumours.

Their penetration into the blood-stream is scanty and irregular and does not lead to the constant and persistent increase of one type of pathological blood cell in the circulation, which is the main characteristic of all the hæmopathies of fowls and other animals.

The hyperplasias are therefore strictly limited to the marrow, where they form pale, sharply defined groupings of undifferentiated cells which may expand and thin the bone. They appear to correspond to myeloma in mammals.

V.—Avian Hæmopathies compared with those of Mammals.

It would be otiose to compare erythromyelosis, leucocythæmia, and lymphocythæmia occurring in fowls with the somewhat similar morbid conditions observed in mammals if, apart from the recognition of a peculiar and possibly quite superficial resemblance, no other contribution could be made to the pathology of a very obscure group of diseases.

It is, however, suggested that the investigation of these hæmopathies in fowls has resulted in observations which explain the origin of the distinctive clinical features of these diseases and moreover confirm some of the recent advances in the knowledge of the hæmatopoietic processes in birds.

These explanations, *mutatis mutandis*, may be applicable to the pathogenesis of several morbid conditions in man and animals.

1. *Oligoerythrocytosis and Poly-erythrocytosis.*

Resemblances and differences between erythromyelosis and progressive oligoerythrocytosis in man have already been discussed in my paper on the fowl-hæmopathy in "Parasitology," 1929.

The question whether human progressive oligoerythrocytosis ("pernicious anæmia") is due to an invisible microbe cannot be yet decided. That, analogically to what is clearly seen in fowl-erythromyelosis, in man the disease originates in the marrow by a vicious production of immature red cells, which are destroyed in the spleen and liver, is an opinion confirmed by numerous observations. This was expressed in other words by Robertson and Rous (1917), as follows:—"The anæmia renders the bone-marrow unable to put forth prope cells, and those it does produce are soon destroyed, thus prolonging

the condition. A similar state of affairs probably exists in many human anæmias."

Evidently not all "anæmias"—primary, secondary, splenic, aplastic, etc.—are explained by the recognition of the genesis of erythromyelosis in fowls. As an example of oligoerythrocytosis due to a visible primary cause not residing in the marrow, one may mention that occurring in rats after splenectomy, as the result of the destruction of erythrocytes in the circulation by the action of the microbe *Bartonella muris*.

2. *Leucocythæmia.*

There is one observation in relation to avian leucocythæmia which has a direct bearing on the analogous conditions in man and domesticated animals: in fowls the morbid process has been traced to one type of immature cell of the granulocyte series. This not only sharply differentiates the diagnosis, but can also be considered as further evidence correlating this hæmopathy with blastogenesis or tumour formation, for most tumours consist of an overwhelming number of cells of one single genetic type. The other resemblances between leucocythæmia and tumours are found in the formation of secondary deposits in both conditions, and these have been repeatedly considered by Piney (1930) and other pathologists.

In fowls we must consider two somewhat similar lesions: leucocythæmias which develop from the leucocytogenetic areas of the marrow and diffuse tumours of the type described by Pentimalli (1915), which to my mind originate from aberrant or persistent areas of marrow-like tissue in other organs. This is confirmed by the fact that in these diffuse tumours the number of white blood cells is scarcely increased, the marrow is little affected, and the largest deposits are found in the liver and other organs.

A revision of the slides of human leucocythæmias in my possession shows that the great majority of the pathological cells can be diagnosed as immature leucocytes, provided the much less numerous cells of the erythroblast type are excluded, whose introduction into the blood-stream is moreover a secondary phenomenon.

3. *Lymphocythaemia.*

The comprehension of the somewhat obscure group of diseases which originate through the more or less intense dissemination of cells of the lymphocytic and reticulo-endothelial groups, is also aided by these observations on fowls; for it can be seen that, notwithstanding great morphological resemblance, cells may function differently and react quite distinguishably to very similar pathological causes.

In this connection the experiments of Korteweg (1929), Richter and MacDowell (1930), are relevant because these authors succeeded in transmitting a lymphocythæmic condition of mice to mice of the same stock; they obtained a variety of manifestations, these differing in their distribution and intensity but not in their nature.

" Neurolymphomatosis gallinarum " has already been alluded to (p. 199) and its resemblance to the disease in mice is evident to observers of both pathological manifestations. The common factor to the diseases in fowls and mice is the abnormal proliferation of cells of the lymphocytic type, whatever their origin and ultimate distribution.

Repeated transmissions in series of these pathological alterations cannot fail in bringing out that the clinical differences are not due so much to variations in etiology as to the situations of the cells which have been induced to multiply ; these situations are influenced by the varying receptiveness of the animal inoculated and possibly also by the mode of infection.

A further study on the comparative pathology of these diseases should therefore help to solve many other difficulties which at present beset the accurate classification of the whole group of hæmopathies and allied conditions.

VI. Summary and Conclusions.

1. As the result of experiments, observations during life, and necropsies, it is concluded that certain specific avian hæmopathies can be classed as : I. Oligoerythrocythæmias. II. Leucocythæmias. III. Lymphocythæmias.

These originate as proliferative conditions of definite tissues in the marrow and therefore might preferably be termed : I. Erythromyelosis. II. Leucomyelosis. III. Myelolymphocytosis.

2. These proliferative conditions of the hæmacytogenic tissues result in the entry of increasing numbers of immature cells into the circulation, throughout the course of the disease. This distinguishes them from many similar ailments which may exhibit an irregular or terminal overflow of immature or pathological cells into the circulation.

3. The oligoerythrocythæmias may originate as the result of many diverse causes, therefore the designation " erythromyelosis " should be restricted to the condition in fowls in which the primary participation of the red bone marrow can be proved, and which display a progressive diminution or lasting excess of erythrocytes.

4. The immature type of the cells predominating in the circulation and the definite lesions in marrow, liver, and spleen, distinguish leucocythaemias and lymphocythæmias from the temporary leucocytosis and lymphocytosis which occur as the result of bacterial infections or injections of chemical irritants of the marrow.

5. The comparison of experimental results after inoculation of blood and organ emulsions of fowls suffering from hæmopathies of myelogenous origin, with the observations made at necropsies of spontaneously similarly diseased birds, confirms the view that where the proliferative process originates in the marrow it leads to the development of the signs of a definite hæmopathy.

6. Where the proliferation originates in other organs or the reticulo-endothelial system, tumour-like appearances, such as secondary deposits (metastases), predominate, and the invasion of the blood-circulation is fitful and limited.

7. In fowls diffuse tumours are at times encountered which appear to originate from aberrant or persistent hæmacytopoietic embryonal tissues outside the marrow, in the inner organs, e.g., the liver. Such rare conditions do show a continuous invasion of the blood with immature cells; it must be understood that they are exceptional.

8. The distinction of leucocythæmia and lymphocythæmia from tumour-like proliferations of persistent hæmatopoietic tissues in the inner organs is helped by comparing the results of blood-examination *in vivo* with the lesions found on necropsy. In diffuse tumours of aberrant hæmatopoietic tissues the numbers of immature cells in the blood are relatively small in proportion to the size of the deposits in different inner organs.

9. The three hæmopathies of fowls described are considered to be due to specific causes and therefore the diseases should not be transmutable. In my experimental experience erythromyelosis always gives rise to erythromyelosis (in clinical terms as oligo-erythrocytosis or polyerythrocytosis) on injection and not any other hæmopathy. The specificity of the cells is maintained, correspondingly to what is observed in tumour transplants.

10. The necropsies on fowls from several different sources in the United Kingdom lead to the same conclusion. The spontaneous hæmopathies observed belong definitely to one of the three groups mentioned; no transitional condition or anything which could be called transitional after careful anatomical analysis was seen in the course of 150 necropsies.

11. When compared with similar diseases in mammals, it is seen that obvious analogies exist in the development of hæmopathies in birds and those of mammals. This is confirmed in relation to allied conditions by recent experimental work with diffuse neuro-lymphomatosis in hens and the somewhat similar disease in mice. It is accordingly to be expected that the pathology of several analogous diseases in mammals will be simplified by attention to the type of cellular unit predominantly involved.

12. Whether there is any possible etiological analogy between the avian diseases and similar hæmopathies in mammals cannot yet be decided, but the observations now made should serve as a guide for experiments in this and other directions.

I should like to express my sincere thanks to Prof. J. Basil Buxton, who very kindly placed the facilities of the Field Laboratory, Cambridge, at my disposal, in connection with the many fowls required for these investigations.

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THE ISOLATION OF TUBERCLE BACILLI FROM CAPTIVE WILD ANIMALS.

By Reginald Lovell,

Division of Bacteriology and Immunology,
London School of Hygiene and Tropical Medicine.

A. S. Griffith (1928 *b*) reviews the subject of tuberculosis in captive wild animals, and draws attention to the importance of knowledge concerning the susceptibility of different species of animals to the different types of tubercle bacillus. He points out that tuberculosis in warm-blooded animals living in the wild state is unknown, or, at least, that no example in which previous association with domesticated animals or human beings could be excluded has been recorded. On the other hand, tuberculosis is the cause of many deaths in animals kept in Zoological Gardens and Menageries. In the present paper details are given of seven strains of tubercle bacilli derived from cases of tuberculosis occurring in the Gardens of the Zoological Society of London.

Technique.

Cultures were obtained by direct methods in two cases, and from the inoculation of original material into guinea-pigs in the remaining five cases. All inoculations both in guinea-pigs and rabbits were given intramuscularly into the left thigh. The pigeons used were inoculated into the muscles of the breast. Smears were taken from all the inoculated animals and acid-fast bacilli found in all the cases with the exception of those returned in the following tables as normal. The cultural characteristics were tested on egg medium, and on inspissated serum with and without the addition of 5 per cent. glycerine, and also on glycerine agar and glycerine potato. The tubes were examined after 21 days at 37° C., and again after further incubation for a similar period at the same temperature. Material for virulence tests was taken from non-glycerinated egg cultures which were always less than 21, and in the case of eugonic strains often only seven, days old.

Details of the Strains of Tubercle Bacilli Examined.

(1) *D.B. No.* 138/28.

Reindeer (*Rangifer tarandus*), born in the Gardens of the Zoological Society and died at the age of 9 months from a non-tuberculous peritonitis due to the perforation of ulcers in the rumen. The autopsy revealed extensive tuberculosis of the lungs. Infected paratracheal glands were used for culturing the virus; microscopical examination of smears from these glands revealed numerous acid-fast bacilli.

ANIMAL EXPERIMENTS.

Animal.	*Material.*	*Result.**	*P.M.*
1. Guinea-pig	0·2 c.c. of gland suspension of Reindeer	K. 46 days ...	Lymphatic glands and spleen enlarged; regional glands caseous.
2. Guinea-pig	0·1 mgm. of 2nd generation culture ...	K. 51 days ...	Local lesion caseous. Lymphatic glands enlarged and caseous. Spleen shows areas of necrosis. Lungs slightly affected.
3. Guinea-pig	0·1 mgm. of 2nd generation culture ...	K. 51 days ...	Local lesion caseous. Lymphatic glands enlarged and caseous. Spleen enlarged and shows areas of necrosis.
1. Rabbit ...	1 mgm. of 2nd generation culture ...	D. 47 days ...	Local lesion caseous. Spleen enlarged with tuberculous nodules. Miliary tuberculosis of lungs.
2. Rabbit ...	1 mgm. of 2nd generation culture ...	K. (moribund) 51 days.	Local lesion caseous. Miliary tuberculosis of lungs and kidneys. Spleen enlarged and caseous. Few nodules in liver. Lymphatic glands enlarged and caseous.
4. Guinea-pig	0·5 c.c. of glandular suspension of guinea-pig No. 1.	K. 53 days ...	Local lesion and regional lymphatic glands caseous. Spleen enlarged, with areas of necrosis.

* D. = Died, K. = Killed.

ANIMAL EXPERIMENTS.

Animal.	*Material.*	*Result.**	*P.M.*
1. Guinea-pig	0·5 c.c. of gland suspension of gnu ...	D. 72 days ...	Local lesion caseous. Regional lymphatic glands enlarged. Spleen and liver show areas of caseation. Lungs only slightly affected.
2. Guinea-pig	0·1 mgm. of 1st generation culture ...	D. 58 days ...	Local lesion caseous mass. Spleen and liver enlarged with caseous areas. Lymphatic glands enlarged and caseous. Lungs moderately affected.
3. Guinea-pig	0·1 mgm. of 1st generation culture ...	D. 59 days ...	Local lesion ulcerated. Spleen enlarged with caseous patches. Extensive areas of caseation in liver. A number of discrete tubercles in lungs. Lymphatic glands enlarged and caseous.
1. Rabbit ...	1 mgm. of 1st generation culture ...	D. 28 days ...	Local lesion caseous mass. Liver, spleen, and lungs miliary tuberculosis. Kidneys moderately affected. Regional lymphatic glands enlarged and caseous.
2. Rabbit ...	1 mg. of 1st generation culture	D. 47 days ...	Local lesion caseous mass. Miliary tuberculosis of lungs. Liver, spleen, and kidneys punctiform tubercles. Regional lymphatic glands caseous.

* D. = Died.

ANIMAL EXPERIMENTS.

Animal.	*Material.*	*Result.**	*P.M.*
1. Guinea-pig	5 mgm. of 10th generation culture ...	D. 33 days ...	Local lesion caseous. Lymphatic glands enlarged and caseous. Spleen and liver show areas of caseation. Miliary tuberculosis of lungs.
2. Guinea-pig	5 mgm. of 10th generation culture ...	D. 36 days ...	Local lesion caseous. Lymphatic glands enlarged and caseous. Spleen and liver show varying sized areas of caseation. Miliary tuberculosis of lungs.
1. Rabbit ...	10 mgm. of 10th generation culture ...	K. 124 days ...	Well nourished. Extensive caseous local lesion. Few tubercles in right lung.
2. Rabbit ...	10 mgm. of 10th generation culture ...	K. 124 days ...	Well nourished. Hæmorrhagic caseous local lesion. Few tubercles in both lungs.
1. Pigeon ...	1 mgm. of 10th generation culture ...	K. 124 days ...	Well nourished and normal.
2. Pigeon ...	1 mgm. of 10th generation culture ...	K. 124 days ...	Well nourished and normal.

* D. = Died. K. = Killed.

Cultural Characteristics.

Typical dygonic growth, with no improvement in growth upon addition of glycerine.

Remarks.—Culturally the virus was of the dysgonic type ; pathogenically, the lesions produced were less severe and extensive than those caused by a bovine virus of standard virulence. It may therefore be regarded as a bovine virus of less than standard virulence.

(2) *D.B. No.* 195/28.

White-tailed Gnu (*Connochætes gnou*), died of pulmonary tuberculosis after 15 years in the Gardens. A few tuberculous foci were present in the kidneys and the bronchial lymphatic glands were caseous.

Cultural Characteristics.

Rather poor growth on egg and serum media, with no improvement on addition of glycerine.

The strain appears to be a bovine virus of standard virulence.

(3) *D.B No.* 262/28.

Grand Eclectus (*Lorius roratus*) is the only bird included in the series. It died from catarrh of the intestine after 18 months in the Gardens. The only tuberculous organ was the liver, which showed minute foci. A culture was obtained by direct methods.

Cultural Characteristics.

Good growth on egg and serum media, showing secondary colonies and producing slight yellowish pigmentation on the serum media and surface growth on the water of condensation. Very little improvement on addition of glycerine to the medium. This strain appears to be a human virus of standard virulence.

(4) *D.B. No.* 306/28.

Two-spotted Palm-Civet (*Nandinia binotata*), died from tuberculosis after only three days in the Gardens of the Society. There was an abscess cavity over the right hip which discharged purulent material containing acid-fast bacilli. The lungs were studded with small greyish-white foci, and the pleural, pericardial, and peritoneal cavities contained excess of fluid showing acid-fast bacilli microscopically. The pancreas and the mesenteric, lumbar, tracheal, sternal, and inguinal glands were enlarged and showed acid-fast bacilli microscopically and, although no abnormality was found in the intestine, the lacteals were prominent and contained many acid-fast bacilli. Material from the lumbar glands was used for cultural purposes, a suspension revealing four or five acid-fast bacilli per field.

Cultural Characteristics.

Good confluent growth on egg and serum media, with heaped-up

ANIMAL EXPERIMENTS.

Animal.	*Material.*	*Result.**	*P.M.*
1. Guinea-pig	0·5 c.c. of gland suspension of civet ...	K. 53 days ...	Local lesion caseous. Regional lymphatic glands slightly caseous.
2. Guinea-pig	0·5 c.c. of gland suspension of guinea-pig No. 1.	K. 43 days ...	Caseous local lesion. Regional lymphatic glands slightly caseous.
3. Guinea-pig	0·5 c.c. of gland suspension of guinea-pig No. 2.	K. 27 days ...	Large caseous local lesion. Regional and sublumbar lymphatic glands caseous, spleen few caseous nodules, liver and lungs few tubercles.
4. Guinea-pig	0·1 mgm. of 1st generation culture ...	K. 93 days ...	No local lesion, occasional lymphatic glands affected, lungs studded with discrete yellowish deposits, of retrogressive type.
5. Guinea-pig	0·1 mgm. of 1st generation culture ...	K. 93 days ...	No local lesion, regional lymphatic glands enlarged; few old standing tuberculous deposits in lungs.
1. Rabbit ...	1 mgm. of 1st generation culture ...	K. 93 days ...	Extensive caseous local lesion. Few discrete yellowish tuberculous deposits in the lungs, one or two in liver.
2. Rabbit ...	1 mgm. of 1st generation culture ...	K. 93 days ...	Extensive caseous local lesion; discrete tuberculous deposits in the lungs.
6. Guinea-pig	1 mgm. of 2nd generation culture ...	D. 57 days ...	Small local lesion. Lymphatic glands enlarged and caseous. Spleen and liver enlarged and tuberculous. Lungs few areas of necrosis.
7. Guinea-pig	1 mgm. of 2nd generation culture ...	D. 77 days ...	Necrotic local lesion. Lymphatic glands enlarged and caseous. Liver and spleen enlarged, with areas of necrosis. Lungs show many discrete tubercles.
8. Guinea-pig	5 mgm. of 12th generation culture ...	D. 39 days ...	Extensive caseous local lesion. Lymphatic glands enlarged and caseous. Liver shows many punctiform tubercles. Spleen enlarged, with areas of necrosis. Miliary tuberculosis of lungs.
9. Guinea-pig	5 mgm. of 12th generation culture ...	D. 42 days ...	Large caseous local lesion. Lymphatic glands enlarged and caseous. Liver slightly affected. Spleen enlarged, with necrotic areas. Miliary tuberculosis of lungs.
3. Rabbit ...	10 mgm. of 12th generation culture ...	K. 124 days ...	Good condition, with extensive local lesion containing thick caseous pus.
4. Rabbit ...	10 mgm. of 12th generation culture ...	K. 124 days ...	Good condition, with extensive caseous local lesion and three or four minute tubercles in the lungs.

* D. = Died. K. = Killed.

secondary colonies. Slight improvement in growth on addition of glycerine to medium. Growth continued on to surface of water of condensation. Slightly brownish growth on glycerine potato.

Remarks.—Culturally this strain is of the eugonic type. Pathogenicity experiments made with early generation cultures indicated that it was of rather less virulence than the standard human type, but experiments made with later generation cultures showed that it possessed a degree of virulence equal to this type. Whether this result may be attributed to the difference in dosage used is doubtful. It may be concluded that the strain is a human virus which on isolation was probably of subnormal virulence, but which later manifested the standard virulence of the human type.

(5) *D.B. No.* 373/28.

Orang-utan (*Simia satyrus*), died from tuberculosis about three or four months after arrival in this country from Sumatra. Her offspring and mate both died from the same disease ; the former was an excellent example of alimentary infection. Post-mortem examination of the mother showed the intestine to be normal. The left lung was collapsed and studded throughout with caseous nodules ; the right lung was solid with large areas of caseation. Both pleural and pericardial cavities contained large quantities of fluid and the heart muscle was atrophied and fibrous. A caseous paratracheal lymphatic gland was used for cultural purposes.

Cultural Characteristics.

Good confluent growth with secondary colonies on egg and serum media. More confluent and rather drier type of growth on addition of glycerine to the medium. Growth continued on to the surface of the water of condensation.

This virus grows similarly to strains of human type, but its virulence for both guinea-pigs and rabbits is below standard, and the lesions to which it gives rise are few and retrogressive.

It would appear to be a human virus of less than standard virulence.

(6) *D.B No.* 963/28.

A Malay Tapir (*Tapirus indicus*), died from tuberculosis of respiratory origin. A tracheo-bronchial lymphatic gland, which microscopically contained only a few acid-fast bacilli, was used for inoculation into a guinea-pig.

Cultural Characteristics.

Moderate growth on egg and serum media, with no evidence of secondary colonies on further incubation. Glycerine added to the medium has no apparent effect upon the growth.

This strain appears to be a bovine virus of standard virulence.

ANIMAL EXPERIMENTS.

Animal.	*Material.*	*Result.**	*P.M.*
1. Guinea-pig	1 c.c. of suspension of gland of orang-utan.	K. 76 days ...	Guinea-pig fat. Regional lymphatic glands enlarged and caseous.
2. Guinea-pig	0·1 mgm. of 1st generation culture ...	K. 93 days ...	Well nourished. Regional lymphatic glands enlarged, with slight caseation.
3. Guinea-pig	0·1 mgm. of 1st generation culture ...	K. 93 days ...	Well nourished. Regional lymphatic glands enlarged.
1. Rabbit ...	1 mgm. of 1st generation culture ...	K. 93 days ...	Normal.
2. Rabbit ...	1 mgm. of 1st generation culture ...	K. 93 days ...	Normal.
1. Pigeon ...	1 mgm. of 3rd generation culture ...	K. 86 days ...	Normal.
2. Pigeon ...	1 mgm. of 3rd generation culture ...	K. 86 days ...	Normal.
4. Guinea-pig	1 mgm. of 3rd generation culture ...	K. 154 days ...	Well nourished. Regional lymphatic glands enlarged and fibrous.
5. Guinea-pig	1 mgm. of 3rd generation culture ...	K. 154 days ...	Well nourished. Regional lymphatic glands enlarged and fibrous. Fibrous areas in spleen and liver.
6. Guinea-pig	5 mgm. of 15th generation culture ...	D. 84 days ...	Trace of caseation at site of inoculation, slight enlargement of regional lymphatic glands and spleen. Cause of death was not tuberculosis, those lesions being insufficient.
7. Guinea-pig	5 mgm. of 15th generation culture ...	K. 124 days ...	Local lesion of thick caseous pus. Regional lymphatic glands enlarged. Liver shows two or three minute tubercles.
3. Rabbit ...	10 mgm. of 15th generation culture ...	K. 124 days ...	Well nourished. Local lesion caseous. Regional lymphatic glands slightly caseous.
4. Rabbit ...	10 mgm. of 15th generation culture ...	K. 124 days ...	Well nourished. Local lesion caseous.

* D. = Died. K. = Killed.

(7) *D.B. No.* 288/29.

A Chimpanzee (*Anthropopithecus troglodytes*) was anæsthetised after having lived in captivity for nearly four years. Post-mortem, there was extensive tuberculosis of the lungs, and miliary tubercles were found in the peritoneum, intestines, liver, spleen, kidneys, and adrenals. A tuberculous mesenteric lymphatic gland was used for cultural purposes.

Cultural Characteristics.

Good confluent growth on egg and serum media with continuation on to the surface of the water of condensation. On further incubation secondary colonies are evident and the addition of glycerine to the medium enhances growth.

There is little doubt from the cultural and animal experiments that this strain is a human virus of standard virulence.

Discussion of Results.

Cultures of tubercle bacilli obtained from wild animals kept in captivity have been studied and their type determined.

Two primates were included in the cases examined, an Orang-utan (*Simia satyrus*) and a Chimpanzee (*Anthropopithecus troglodytes*). Both furnished strains of the human type ; one was of standard virulence for the animals tested, the other was of definitely subnormal virulence. Although experimental evidence has been produced (Cobbett, 1917) to show that monkeys of all varieties are equally susceptible to tubercle bacilli of human and bovine type, yet the greater proportion of tuberculous infections acquired naturally are caused by the human type. This is doubtless to be ascribed to the greater frequency with which monkeys in captivity are exposed to infection with this type. A. S. Griffith (1928 *b*) records that, of 30 primates, 22 were affected with the human type of tubercle bacillus and eight with the bovine.

The only member of the Order *Carnivora* examined was a Two-spotted Palm-Civet (*Nandinia binotata*) and a human type of virus was recovered, which, however, appeared to be of less than standard virulence. Of the two members of this order examined by A. S. Griffith (1928 *b*), one was infected with a bovine, the other with a human, type of virus.

Amongst the *Artiodactyla* a White-tailed Gnu (*Connochætes gnou*) was found to be infected with a bovine virus of standard virulence, and a Reindeer (*Rangifer tarandus*) with a bovine virus of less than standard virulence. Of the order *Perissodactyla*, a Malay Tapir (*Tapirus indicus*) was examined ; this animal was infected with a bovine virus of standard virulence. Of 15 cases of tuberculosis amongst Ungulata (which includes both *Artiodactyla* and *Perissodactyla*) mentioned by A. S. Griffith (1928 *b*), eleven were of the bovine and four of the human type.

Only one bird—a Grand Eclectus (*Lorius roratus*)—of the order *Psittaciformes* was examined ; the infection proved to be due to a

ANIMAL EXPERIMENTS.

Animal.	*Material.*	*Result.**	*P.M.*
1. Guinea-pig	0·5 c.c. of gland suspension of tapir ...	K. 69 days ...	Extensive caseous local lesion. Lymphatic glands enlarged and caseous. Spleen and liver show areas of caseation.
2. Guinea-pig	1 mgm. of 9th generation culture ...	D. 14 days ...	Large caseous local lesion. Regional lymphatic glands enlarged and caseous.
3. Guinea-pig	1 mgm. of 9th generation culture ...	D. 25 days ...	Large caseous local lesion. Lymphatic glands enlarged and caseous. Liver and spleen show areas of necrosis. Punctiform hæmorrhages in the lungs.
1. Rabbit ...	10 mgm. of 9th generation culture ...	D. 59 days ...	Very extensive caseous local lesion. Liver, spleen, and kidneys show tuberculous deposits. Lungs converted into tuberculous mass. Lymphatic glands enlarged and caseous. Tubercles in omentum, intercostal spaces, and intestinal wall.
2. Rabbit ...	10 mgm. of 9th generation culture ...	D. 74 days ...	Very extensive caseous local lesion. Liver and kidneys show minute tubercles. Lungs converted into caseous and calcified mass. Lymphatic glands enlarged and caseous.

ANIMAL EXPERIMENTS.

Animal.	*Material.*	*Result.**	*P.M.*
1. Guinea-pig	1 mgm. of 6th generation culture ...	D. 36 days ...	Small caseous local lesion. Lymphatic glands enlarged and caseous. Spleen and liver show areas of necrosis. Lungs show areas of consolidation and necrosis.
2. Guinea-pig	1 mgm. of 6th generation culture ...	D. 37 days ...	Small caseous local lesion. Lymphatic glands enlarged and caseous. Spleen and liver show areas of necrosis. Miliary tuberculosis of lungs.
1. Rabbit ...	10 mgm. of 6th generation culture ...	K. 123 days ...	Caseous local lesion. Few tubercles in lungs.
2. Rabbit ...	10 mgm. of 6th generation culture ...	K. 123 days ...	Caseous local lesion. Regional lymphatic glands caseous. Left lung collapsed and 5 c.c. of fluid in pleural cavity, but no acid-fast bacteria found in fluid.

* D. = Died. K. = Killed.

human virus of standard virulence. Infection of parrots with tubercle bacilli of this type has been noted by Cobbett (1917), and more recently cases have been recorded by Stableforth (1929) and Martinaglia (1929). Of the 17 various birds examined by A. S. Griffith (1928 *b*), the strains of tubercle bacilli obtained all proved to be of the avian type.

Of the seven strains here recorded, three appeared to be of rather lower virulence than that normal to their type. It is well known that, apart from lupus in the human subject, the most fruitful source of attenuated strains has been the horse. Thus F. Griffith (1911) studied five equine strains, and found that two were attenuated. M'Fadyean (1924) reported on eleven strains. A. S. Griffith (1928 *a*), in a critical review, considered four out of eight of these to be of modified virulence ; the remaining three strains were not subjected to animal experimentation. A. S. Griffith himself examined eight strains, and of these five were of low virulence. Of the 13 recorded by Stableforth (1929) six were attenuated.

In order to ascertain whether the high proportion of attenuated strains met with in the present series of experiments was purely fortuitous, it was decided to examine the literature dealing with cases of tuberculosis in captive wild animals. A. S. Griffith (1916-17 *b*) found that, of ten strains isolated from tuberculous monkeys, one bovine and one human strain were attenuated. In his study of 37 cases (1928 *b*) in captive wild animals, it is clear that Griffith encountered many strains which were of subnormal virulence. Excluding three eugonic strains from monkeys, the virulence experiments of which are not given in detail, and one strain of avian type, there appear to be two out of 18 bovine viruses and six out of 15 human viruses which showed some degree of attenuation.

The following table comprises the collected figures of strains from horses and captive wild animals.

TABLE I.

	Type of Tubercle Bacillus.			
	Human		*Bovine.*	
	Standard Virulence.	*Sub-standard Virulence.*	*Standard Virulence.*	*Sub-standard Virulence.*
Horses	—	—	17	17
Captive Wild Animals.	15	9	22	4

(Figures collected from :—Griffith, F. (1911), five horses ; M'Fadyean (1924), eight horses ; Griffith, A. S. (1928 *a*), eight horses ; Stableforth, A. W. (1929), thirteen horses ; Griffith, A. S. (1916-17 *b*), ten monkeys ; Griffith, A. S. (1928 *b*), 33 captive wild animals ; and seven here recorded.)

The high proportion of attenuated strains in these animals is in marked contrast with those isolated from domesticated cattle and pigs. No such attenuated bacilli were encountered in the investigation of 52 cases from cattle and 25 from pigs (A. S. Griffith, 1928 *a*). Experience of the injection of guinea-pigs with milk from tuberculous cows

has shown with what extreme rarity aberrant strains are encountered. The same holds true of tubercle bacilli of human origin (excluding lupus). 212 viruses were isolated from samples of human sputum by A. S. Griffith (1916), and although four were culturally atypical yet none was of modified virulence. Only one attenuated strain was isolated from 141 cases of human bone and joint tuberculosis by A. S. Griffith (1916-17 *a*), although four others were culturally atypical.

The proportion of avirulent strains of tubercle bacilli isolated from human and bovine cases would therefore appear to be much lower than that isolated from horses and captive wild animals. The significance of this observation is difficult to assess. Most strains of lowered virulence hitherto recorded have been isolated from a relatively chronic and often localised type of disease. It would appear, however, judging from the limited number of cases available, that in captive wild animals a strain that is capable of giving rise to generalised tuberculosis in the host may yet prove of comparatively low virulence for the guinea-pig. The mechanism by which attenuation may occur within the body is entirely unknown, and it would serve no useful purpose to discuss that question at the moment.

Summary.

1. The type of infecting bacillus was determined in seven captive wild animals (including one bird). Three bovine and four human strains of tubercle bacilli were recorded. One of the bovine and two of the human strains appeared to be of less than standard virulence.

2. Attention is drawn to the fact that in lupus, and in tuberculosis of horses and of captive wild animals, a considerable proportion of strains isolated have been of subnormal virulence. This finding is in marked contrast with the almost uniformly high virulence of strains isolated from other tuberculous lesions in man, and from tuberculous cows and pigs.

My thanks are due to Dr. A. S. Griffith for his help and advice ; and to Col. A. E. Hamerton, of the Zoological Society of London, for permission to quote from pathological reports.

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OBSERVATIONS REGARDING THE ETIOLOGY OF ACTINOMYCOSIS IN CATTLE AND SWINE.

By G. O. Davies, B.V.Sc., M.R.C.V.S., D.V.H.,
and
H. L. Torrance, M.R.C.V.S., D.V.S.M.
(*Department of Veterinary Pathology, University of Liverpool.*)

The frequent occurrence in Irish cattle of lesions bearing a slight resemblance to tuberculosis, and the presence of other pathological processes which could not be attributed to any known cause, led the authors to a systematic investigation of such cases. The material together with the post-mortem observations were collected by the second author (H.T.) and sent to the first author (G.D.) for laboratory investigation. As the conditions were only encountered on post-mortem examination, it is unfortunate that in some cases a complete examination of the whole carcase and offal was not possible.

The writers record their observations on twenty such cases. It was their intention to continue the investigation until a much larger number of cases had been dealt with, but, owing to a change of appointment on the part of one of the writers and the consequent impossibility of continuing the work, it was deemed advisable to publish our findings in their present state.

The distribution and the macroscopic appearances of the various lesions have been noted, and diagnosis was based on the result of histological, bacteriological, and serological examinations, the particulars of which will be detailed later.

Every chronic inflammatory condition of a suppurative character presenting the well known granules in the pus has until recently been regarded as actinomycosis. The presence of these granules, which could be detected by naked eye examination, was the criterion for diagnosis, inasmuch as earlier investigators had shown that these club-bearing granules were colonies of the Actinomyces or "ray fungus." The condition has been recognised in cattle since 1877, when it was described by Bollinger. Boström in 1890 described the cause to be a streptothrix which grew readily on ordinary media under ærobic conditions, but Wolff and Israël in 1891 described the form more commonly met with in Britain and the one which has been encountered by most investigators. The *Streptothrix israëli*, as it is often called, is much more exacting in its cultural requirements and grows but very slowly under a reduced oxygen tension. In 1902 Lignières and Spitz, working in South America, isolated a short oval Gram-negative bacillus from actinomycotic lesions in soft tissues, but at the time little attention was paid to their findings in Britain. This was, no doubt, partly due to the fact that the streptothrix had been accepted as the etiological factor concerned in all cases of actinomycosis and partly also to the reason given by Magnusson, that actinobacillosis as described by Lignières and Spitz seemed

to be an epizootic peculiar to the Argentine. Furthermore, actinomysosis had come to be regarded as an affection of the head involving chiefly the tongue or jaw, and it is conceivable also that lesions in the glands and internal organs, which are now attributed to actinobacillosis, were then either overlooked in ignorance or placed in the category of tuberculosis. The feasibility of the latter will be exemplified later.

Numerous investigators, including Griffith and Bosworth in this country, have confirmed the findings of Lignières and Spitz. More recently we have the excellent article by Magnusson, detailing his observations on 135 cases in cattle and 273 cases in pigs, and summarising the position at that date.

Actinomycosis can no longer be considered a pathological entity. It is now known that the clinical condition so named, which is characterised by a granulomatous lesion in soft tissues and by a rarefying ostitis in bones, together with the presence of macroscopic granules in the pus, is no longer a specific disease. The property of producing such lesions is possessed by several organisms, and they can roughly be classified according to the lesions which they produce and the organs in which they are found. Consequently, we use the term actinomycosis in its broadest sense, as indicating a clinical condition only.

The following causal organisms have been described :—

1.—*Actinobacillus lignièresi* (Lignières and Spitz, 1902). It may be accepted as a general rule that actinomycosis in bovines primarily affecting the soft parts of the head, such as the tongue, lips, gums, palate, and neighbouring lymphatic glands, provided bone tissue is left intact, is caused by the actinobacillus.

2.—*Streptothrix actinomyces* (Israël and Wolff, 1891). Actinomycosis in bovines involving the jaw-bone or any other bone and soft tissues as well is caused by the streptothrix. In pigs the fungus is responsible for approximately 70 per cent. of mammary gland actinomycosis.

3.—*Staphylococcus pyogenes* is responsible for apparently all cases of udder actinomycosis in cows and for about 15 per cent. mammary gland lesions in sows.

4.—*Corynebacterium pyogenes* has been associated with actinomycotic lesions, but it is uncertain at present whether this organism alone is responsible for the lesions in which it has been found.

A systematic laboratory examination was made of twenty-one cases which presented lesions of an actinomycotic nature, but as circumstances prevented one case from being properly dealt with the details of only twenty are recorded.

In order to facilitate a general survey of the cases they have been arranged in tabular form (*see* Table No. 1). The lesions recorded represent all the macroscopic lesions which were apparent on examination, but in some cases, after detecting the lesion in one organ, it was not possible to identify the associated carcase. This applied more particularly to tracing the carcases associated with diseased tongues.

TABLE I.

SUMMARY OF CASES EXAMINED INDICATING LESIONS AND FINDINGS.

Case.	*Tongue A.*	*Hard Palate B.*	*Lymph Glands. C.*	*Liver. D.*	*Stomach. E.*	*Other Organs. F.*	*Organism Isolated.*	*Pathological Findings.*
B. 1.			Ant. mediastinal (1) Portal (2)	Lesion		Peritonitis	Actinobacillus from D.	Actinobacillosis
B. 2.			Lt. submaxillary (1) Lt. pharyngeal (2)				Actinobacillus from C. (1)	Actinobacillosis
B. 3.	Lesion						Actinobacillus from A.	Actinobacillosis
B. 4.		Lesion	Lt. submaxillary (1) Rt. submaxillary (2) Pharyngeal (both) (3) Parotid (both) (4)			Cheek	Actinobacillus from B., C. (1 and 2) and F.	Actinobacillosis
B. 5.	Lesion		Lt. pharyngeal (1) Rt. pharyngeal (2)				Actinobacillus from A. and C. (2)	Actinobacillosis A. and C. Tuberculosis C. (1)
B. 6.	Lesion		Submaxillary				Actinobacillus from A. and C.	Actinobacillosis
B. 7.		Lesion	Pharyngeal (both)				Actinobacillus from B. and C.	Actinobacillosis
B. 8.			Rt. xiphoid	Lesion		Peritonitis	Actinobacillus from D.	Actinobacillosis

B. 9.	Lesion		Rt. pharyngeal (1) Lt. pharyngeal (2)	Lesion		Rt. cardiac lobe lung	Actinobacillus from A., C. (2) and F.	Actinobacillosis Tuberculosis C. (1)
B. 10.	Lesion						Actinobacillus from A.	Actinobacillosis
B. 11.	Lesion						Actinobacillus from A.	Actinobacillosis
B. 12.			Portal and dorsal	Lesion				Tuberculosis
B. 13.						Udder (pig)	Streptothrix actinomyces from F.	Actinomycosis
B. 14.					Rumen	Peritonitis (1) Pleurisy (2)	Actinobacillus from E.	Actinobacillosis
B. 15.						Pleurisy (1) Peritonitis (2)		Tuberculosis
B. 16.								Not investigated
B. 17.			Mediastinal	Lesion	Rumen	Oesophagus	Actinobacillus from D., E., and F.	Actinobacillosis
B. 18.					Rumen		Actinobacillus from E.	Actinobacillosis
B. 19.		Lesion	Lt. submaxillary				Actinobacillosis from B. and C.	Actinobacillosis
B. 20.					Reticulum		B. pyogenes from E.	Confirmed
B. 21.			Intercostal		Reticulum	Diaphragm	Actinobacillus from C. and E.	Actinobacillosis

It will be seen from the table that the cases can be arranged in four groups, according to the organisms responsible for the pathological processes.

Group 1. Actinobacillus isolated alone in 14 cases.

Group 2. Actinobacillosis and tuberculosis coexisted in two cases.

Group 3. Streptothrix actinomyces isolated in one case.

Group 4. Corynebacterium pyogenes isolated in one case.

Group 5. Tuberculosis present alone in two cases.

All the material investigated with the exception of Case P. 13 was of bovine origin. Case P. 13 from a pig was the only case from which the *Streptothrix actinomyces* was isolated. The actinobacillus was the only organism isolated from cases of apparent actinomycosis in cattle, but no lesions involving bone were forthcoming for investigation. The cases of tuberculosis are included inasmuch as they presented lesions which could not, with any degree of certainty, be differentiated from actinomycosis by naked eye examination. One case is recorded where the actinobacillus was obtained in pure culture from a tongue lesion and the right retropharyngeal lymphatic gland. Attempts to grow the organism from the abnormal left retropharyngeal gland failed, but this was accounted for when a microscopical examination of a section was made. The section revealed the typical histological features associated with tubercle, and acid-fast bacilli could be seen when sections were appropriately stained. *Corynebacterium pyogenes* was the organism responsible for case B. 20, when a polypoid lesion in the reticulum was the only lesion present. Apart from its inclusion in the table, no further reference will be made to this case, as it has already been placed on record in this Journal.*

In the course of this article our attention will be confined chiefly to the sixteen cases of actinobacillosis in bovines and to actinomycosis of the mammary gland of a pig.

Actinobacillus lignieresi.

Actinobacillus lignièresi was isolated in culture from no fewer than twenty-eight different tissues. The lymphatic glands yielded cultures in nine instances, the tongue in six, stomach in four, hard palate and liver in three, and the lungs, œsophagus, and the cheek in one case.

Macroscopic Appearances.

The general naked eye appearances of the lesions conform very closely to the descriptions which have been given in the numerous publications on this subject. The presence of pus with the characteristic granules was not a constant feature in our cases, but perhaps we may attribute this to the fact that most of our cases were " young." Nevertheless, it was apparent that the consistency of the nodules varied considerably, suggesting that as the age of the lesions increased the tendency for the centres to break down became more evident.

* Vol. 43, 1930, p. 147.

This was particularly noticeable in lesions of different sizes situated in the lung substance. The smaller lesions when incised showed a distinct convexity of the cut surface but no pus, whereas in the larger ones pus was very evident, and on pressure a further quantity exuded from the interstices. The breaking down of the older lesions would appear to be due to (1) the progressively increasing tension within the capsule, reducing the blood supply, and (2) the organismal activity within the lesion.

A striking feature of the larger lung lesions was the deposition of a greenish black pigment, which radiated in rough irregular lines from the centre.

Affected lymphatic glands were larger than normal and of a firmer consistency. On section small yellowish glistening nodules could be seen which had rather a characteristic arrangement. We invariably found that they were arranged in the peripheral portion of the cortex, and in the majority of cases in irregular clusters towards one end of the gland. It has been our experience that they could be easily missed if attention was paid to one particular part of the gland or even if one relied on one incision. These nodules appeared like pellets of granulation tissue in a fibrous capsule, and in some cases the contents could be enucleated under pressure.

Similar nodules were found in lesions in other organs of the body, the general appearance being very like shot embedded in a fibrous connective tissue. In the tongue and hard palate these lesions were occasionally accompanied by a superficial ulceration, at the depth of which one could occasionally discern these actinobacillary nodules.

In Case B. 1 a very marked peritonitis was associated with the liver lesion, and a section through the liver revealed a very distinct layer of nodules lying on the surface of the liver and In the depth of the exudate.

Where actinobacillosis was confirmed in the reticulum, rumen, and œsophagus, the condition was accompanied by a very marked thickening of the tissue, in which the previously mentioned nodules were to be found in large numbers.

A similar diffuse thickening was encountered in the hard palate and cheek, but in the former there was as a rule very little to be seen superficially which would indicate the extent to which the infection existed in the depth of the tissues.

Histology.

Following a naked eye examination, the diseased tissues were apportioned for histological and cultural examination. Sections were prepared by the paraffin method and were stained in various ways, the chief methods adopted being Gram, Plaut, hæmatoxylin and eosin, and Loeffler's alkaline methylene blue.

A detailed account of the histological features is not called for, as, apart from a few minor differences, the appearances resemble very closely the classical picture which has been so often described under streptothrix infections.

The club bearing granules are usually very numerous and scattered

throughout the affected tissue. There are no central radiating filaments, but their place is taken by clusters of small Gram-negative cocco-bacilli. These may occupy the centre of the smaller colonies, but in the larger ones the centre is often structureless with the organisms forming a zone midway between this centre and the peripheral layer of clubs. The very well-defined clubs are very much larger than in streptothrix lesions and sometimes occupy half the area of colony. Bacilli are often seen between the clubs.

These colonies are usually embedded in granulation tissue, which gradually becomes more fibrous towards the periphery, where a definite capsule is evident. The leucocytic infiltration is not such a prominent feature as in streptothrix sections. Giant cells are often present.

Isolation of the Organism.

Cultures were attempted from the majority of diseased tissues, and in some of the cases the actinobacillus was obtained in pure culture from as many as three or four different organs in the same case.

The possibility of attempting cultures from granules in the pus was in the majority of cases impracticable, as pus was by no means a constant feature. Nevertheless, little or no difficulty was experienced in procuring pure cultures from diseased tissues. The method adopted was to sear the surface of the tissue, and then with sterile instruments to section the tissue and pick out the nodules. Cultures were often obtained by merely expressing a lymph-like fluid from the affected tissue and sowing on to ordinary media. In other cases one or more nodules were held with a fine pair of sterile forceps and reduced to a consistency of pulp by repeatedly cutting with sterile scissors. This was then sown under both ærobic and anærobic conditions.

Morphological and Cultural Characters.

Actinobacillus lignieresi is a short round-ended bacillus, which may be described as a cocco-bacillus. The organism is pleomorphic, coccal forms as well as long bacillary forms being usually present. The microscopic appearance varies considerably according to the medium on which it has been grown and the length of time during which it has been subcultured.

The organism stains readily with ordinary aniline dyes but does not retain Gram stain. It is non-motile.

It grows readily on ordinary media under ærobic conditions, being also a facultative anærobe. A more luxuriant growth is obtained if blood or serum is added to the medium, but the addition of glucose although not preventing growth seems to retard it.

In primary culture on agar or nasgar the bacillus appears in less than 24 hours in the form of small discrete, bluish, translucent colonies. On subculture a more opaque uniform film appears, the organism displaying a tendency to spread. The actinobacillus grows very quickly and subcultures usually show evidence of growth in six to ten hours. Broth is a very suitable medium, and the bacillus

produces a turbidity together with a surface pellicle. Small suspended floculi are also apparent, but these tend to settle and after a few days a copious sediment is evident. There is no liquefaction of solid serum or gelatin. The growth in the latter resembles that in broth when it is grown at about 37° C. Indol is not produced in peptone water, and, apart from a slight acidity, there is little or no change in litmus milk. The organism does not live long in culture, and we have found it necessary to make subcultures every four or five days in order to avoid losing the strain. Its heat resistance is not very marked ; it was alive after five minutes but dead after ten minutes' exposure at 60° C.

The fermentation reactions of most of our strains were tried. The method employed was the one referred to by Magnusson, in which the base was bouillon made from Liebig's meat extract, to which the sugars were added in ½ per cent. quantities. The indicator was bromthymol blue. Several sugars were fermented, but gas production was absent in all the tubes. Acid was produced in Lactose, Glucose, Lævulose, Maltose, Saccharose, Mannite, Xylose and Galactose. The following sugars remained unchanged, Salicin, Sorbite, Inulin, Adonite, Inosite, and Dulcite.

Agglutination.

The statement made by Magnusson (1) that " agglutination as a means of diagnosis is undoubtedly a safe method," prompted us to use this method in order to confirm our diagnosis. We were not only successful in doing this, but were also able to show that all the strains tested were serologically identical.

A rabbit was inoculated intravenously with gradually increasing doses of *Actinobacillus lignièresi* (Case B. 7) suspended in sterile saline solution. Five inoculations were given at weekly intervals before the serum was tested. The serum from the rabbit taken prior to being immunised showed slight agglutination at a dilution of 1 : 20, but after immunisation water clear results were evident at a titre of 1 : 640.

Having thus procured an antiserum against the actinobacillus, emulsions of all strains were prepared as soon as they were isolated. Control emulsions were also used. These consisted of secondary organisms which grew in culture alongside the actinobacillus. A further proof of the reliability of the serum was given by the repeated negative results which followed attempts to associate secondary organisms with the lesions met with in Case B. 12. Having at first concluded that we had missed the specific organism, we were interested to find on microscopical examination of sections that the lesions were due to tuberculosis and not to the actinobacillus.

Eleven strains of the actinobacillus were used as antigens in agglutination tests with this antiserum. All agglutinated at a titre of 1 : 640 and one antigen (Case 19 C.) at a titre of 1 : 1280.

Pathogenicity.

Only two attempts were made to ascertain the virulence of the

bacillus for guinea-pigs. Intraperitoneal inoculations of half a twenty-four-hour agar culture suspension of Case 1, and of a whole agar culture suspension of Case 5, were both followed by negative results.

Streptothrix Actinomyces.

Only one of the cases studied resulted from infection with *Streptothrix actinomyces*, and this occurred in a sow. We failed to isolate the streptothrix from any case of bovine origin.

Case P. 13 was the udder of a sow which revealed a certain amount of fibrosis, in the substance of which were numerous small abscesses about the size of a hazel nut. In addition there were several small glistening nodules which appeared to involve the subcutaneous tissue. There was no apparent softening in the centre of these nodules.

Histology.

Sections were prepared and stained as in the previous cases. There was a general groundwork of fibrous connective tissue in which were to be seen numerous large colonies surrounded by a very marked capsule. The colonies consisted of a mass of branching filaments which revealed a very marked granular appearance. These granules were very clearly defined, and arranged transversely like the rungs of a ladder, so much so, that one was at first led to think that they were bacillary forms arranged in palisade fashion. The extreme regularity of their arrangement, however, did not support this view.

These filamentous colonies were not surrounded by the definite peripheral zone of clubs which one usually sees in actinomycosis. Nevertheless, occasional clubs were present, and it bears out Magnusson's statement with regard to the disease in pigs that " the clubs on the actinomycosis granules are seldom or never so well petioled and clearly defined as, for instance, in streptothrix infection in cattle." The unusual appearance of these sections led us to seek further confirmation, and sections were sent to Dr. Magnusson, who very kindly examined them and reported that they were typical of actinomycosis in swine due to *Streptothrix israëli.*

Isolation of the Streptothrix.

Attempts to isolate the causal organism from the nodules were conducted in precisely the same way as was detailed under *Actinobacillus lignièresi.*

Surface cultures in twenty-four hours revealed a mixed growth of Gram-negative coliform organisms and staphylococci, but nothing was evident in deep glucose agar until the fourth day. The latter colonies presented the typical appearance associated with primary cultures of the streptothrix. Further examination was deferred until the seventh day. Gram-positive short wavy filaments, together with oval, coccal, and bacillary forms, were present in stained films.

Occasional filaments revealed dichotomous branching, and a breaking up of the filaments into granules was very evident. Subcultures were made and grown under anærobic conditions.

Morphological and Cultural Characters.

The organism was much more difficult to grow than the actinobacillus and required a reduced oxygen tension. It grew well in shake culture in serum glucose agar, and surface cultures were also obtained under anærobic conditions. We succeeded in getting good surface cultures by growing on serum glucose agar in a closed jar in the presence of *B. subtilis*, but our best results followed the use of a closed jar in which the air was replaced by coal gas.

In deep serum glucose agar, the growth appears as a zone of small colonies about half an inch below the surface of the medium after about three or four days incubation. Cultures were also obtained in serum glucose broth under ærobic conditions.

Surface cultures are obtainable on serum glucose agar when grown under usual anærobic conditions or in an atmosphere of CO_2. As in shake cultures, colonies do not appear until the third or fourth day. They are minute, clear, and resemble dewdrops, but as incubation continues they become opaque white, and later assume a faint brownish colour. The colonies with their raised centres present a very characteristic appearance under low magnifications.

Discussion.

Although our examinations have not been very extensive, we have sufficient evidence to confirm the findings of other writers, that clinical actinomycosis can no longer be regarded as a pathological process attributable to any one bacterial agent. The *Actinobacillus lignièresi* was responsible for the disease in cattle and the streptothrix actinomyces for a case of actinomycosis in the udder of a sow. The relative importance of actinobacillosis in cattle is further emphasised by the fact that our observations are based on consecutive and not on selected cases.

We have failed to isolate the streptothrix from cases of actinomycosis in the tongue, and are thus far in complete agreement with Magnusson, who states " From what we now know, there is simply no actinomycosis of the tongue in cattle, the affection being always actinobacillosis of the tongue." Also with reference to the frequency in which one finds actinobacillary lesions in the lymphatic glands, he states that " if lymphatic glands are involved we may be sure that the causative agent is not *Streptothrix israëli* but *Actinobacillus lignièresi*."

The actinobacillus can also be held responsible for numerous lesions in the internal organs, some of which are macroscopically indistinguishable from tuberculosis. The distribution of the lesions displays a further similarity to lesions of a tuberculous origin, and the frequency with which lesions of the larger organs are accompanied by lesions in the associated lymphatic glands often makes a differential diagnosis by that means difficult.

The cases presenting the greatest difficulty in macroscopic differential diagnosis are certain cases of tuberculosis of the liver and acute tuberculous pleurisy and peritonitis. In the former, where tuberculous lesions are found in some other part of the body they may assist in the diagnosis, but when the only apparent lesions present are in the substance of the liver and the portal lymphatic gland, a not infrequent condition, recourse to microscopical examination to distinguish the two conditions will frequently be necessary. The differential diagnosis is of considerable importance in meat inspection, as it is well known that where tuberculous lesions exist in the substance of the liver all the organs and their associated lymphatic glands, together with the carcase lymphatic glands, must be examined for evidence of tuberculosis, whereas if the condition is actinobacillosis and the liver only is affected it is unnecessary to examine all the carcase lymphatic glands.

Acute tuberculous pleurisy and peritonitis with no tuberculous lesions showing in the lymphatic glands also present considerable difficulty, because in both cases the exudate on the serous membranes contains a large number of very minute, shot-like yellow nodules. Again, to distinguish definitely the two conditions microscopical methods are necessary, and in meat inspection this procedure is essential, as it is customary to condemn the whole carcase and offal of an animal suffering from acute, diffuse, tuberculous pleurisy and peritonitis.

In one of our cases (B. 2) the only lesions present were in the submaxillary and retropharyngeal lymphatic glands, which indicates the necessity in meat inspection, even when no lesions are found in the tongue, palate, or cheeks, of making multiple incisions into the submaxillary and retropharyngeal lymphatic glands as a routine procedure.

The position and distribution of the lesions indicate very strongly that the channel of infection is by way of an injured mucous membrane. The organs most frequently affected are the tongue, hard palate and cheeks, reticulum, rumen, and œsophagus ; and where other parts are involved, such as liver, diaphragm, peritoneum, lungs, and pleura, the oldest lesions are generally to be found involving the mucous membrane. Injury to the bovine digestive tract by foreign bodies is so common that there is ample opportunity for infection to occur by this means, and where the injury is slight, such as occurs when the mucous membrane is simply abraded or where a slender and sharp foreign body penetrates the stomach wall and returns to the intestine, the conditions are ideal for the development of the infection.

Once the disease is established in a tissue it would appear to spread by way of the lymphatic vessels and glands, and by contiguity.

The liver and lungs are frequently involved with the diaphragm in cases where the infection has entered by the reticulum and spread by simple contiguity.

Summary and Conclusions.

Actinobacillosis is a common disease of Irish cattle.

Although the tongue is the commonest seat of disease, the hard palate and cheeks are often affected, and not infrequently the abdominal and thoracic organs and serous membranes.

Clinical actinomycosis of the soft tissues, such as tongue, lymphatic glands, and internal organs, when unaccompanied by bone lesions is caused by *Actinobacillus lignièresi.*

The *Streptothrix actinomyces* has been isolated from a case of actinomycosis in the udder of a sow.

Cattle presenting extensive lesions of the internal organs on post-mortem examination appeared perfectly healthy immediately prior to slaughter. Many of them were good fat bullocks.

In certain cases it is impossible to distinguish actinobacillosis from tuberculosis without a microscopical examination ; a definite diagnosis has a practical bearing on meat inspection.

The channel of infection would appear to be through a wound in the skin or a mucous membrane.

The agglutination test is a valuable diagnostic method for the identification of the actinobacillus.

The writers wish to thank Professor Gaiger, F.R.C.V.S., for his interest and advice in the course of the investigation.

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Details of Cases.

Case B. 1.

Subject.—An Irish bullock in very good condition, slaughtered on May 20th, 1929.

Lesions.—The lungs were normal. The parietal pleura had been " stripped," presumably on account of pleurisy, and unfortunately was not presented with the offal and carcase for examination. The mediastinal lymphatic glands were enlarged, pale, and firmer in texture. Numerous shot-like pale yellow granules could be seen arranged round the periphery of the gland, with a cluster posteriorly.

The bronchial lymphatic glands were enlarged and pale but showed no nodules.

The liver was slightly cirrhotic from fluke infestation.

The lateral half of the left portion of the liver was one solid mass of fibrous tissue, embedded in which were a large number of small, round, firm, yellow nodules. On section there was no pus. Near to this mass there were a few nodules distributed irregularly in the liver substance.

A very striking appearance was produced by a layer of numerous, very small, shot-like, yellow nodules embedded in an exudate on the peritoneal surface of the liver.

The portal lymphatic gland was enlarged and pale, and showed numerous nodules.

Stomach. The peritoneal surface of a large portion of the rumen and reticulum was covered by a similar exudate to that seen on the liver.

Spleen. Peritoneal exudate as described.

Kidneys. Pale, with some petechiæ.

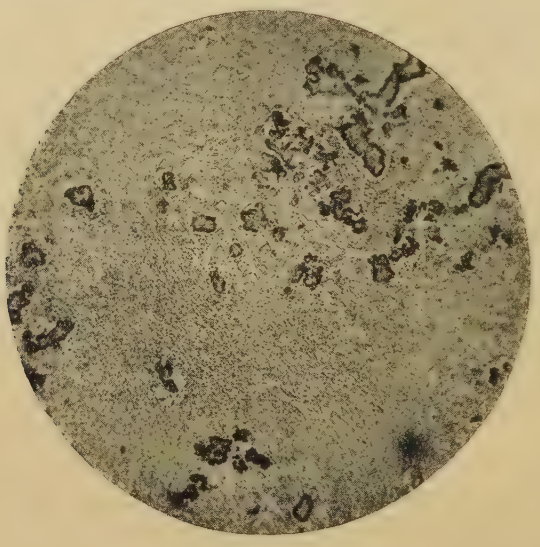

FIG. 1.

Section from submaxillary gland. Case B. 2 (× 50). Shows numerous small colonies of the actinobacillus. The clubs, which are deeply stained, define the boundaries of the colonies.

Case B. 2.

Subject.—A four-year-old Irish bullock slaughtered on June 9th, 1929.

Lesions.—The left submaxillary and left retropharyngeal glands only were affected. The former presented numerous shot-like yellow nodules situated chiefly in the periphery of the gland, while the latter showed only one nodule.

There were no lesions in the tongue.

Case B. 3.

Subject.—A four-year-old Irish Bullock slaughtered on June 9th, 1929.

Lesions.—Only the tongue affected, the lymphatic glands in the neighbourhood appeared normal. The dorso-lateral aspect of the dorsum, particularly on the right side, presented numerous typical nodules. Each nodule was small, up to the size of a pea, round, firm in texture, pale yellow in colour, with some more deeply coloured spots which were only noticeable on very close inspection.

Case B. 4.

Subject.—A 3½-year-old Irish bullock slaughtered on June 9th, 1929.

Lesions.—There were no lesions in the tongue. Both submaxillary, retropharyngeal, and parotid lymphatic glands were affected, being enlarged

and containing a large number of typical shot-like nodules, arranged particularly round the periphery of the glands.

The hard palate contained an enormous number of nodules, like small yellow round seeds, which, on careful inspection, could be seen shining through the mucous membrane.

A similar picture was apparent on the buccal mucous membrane, but here the nodules were a little bigger. The hard palate, when removed, was found to be considerably thickened from the enormous number of typical nodules it contained.

In the depths of the buccal mucous membrane the lesions were not so numerous, nor so uniform in size.

Case B. 5.

Subject.—An Irish heifer, one year and nine months old, slaughtered on June 18th, 1929.

Lesions.—Tongue. Numerous nodules and small brownish areas could be seen where the lesions had "ulcerated," particularly on the dorso-lateral aspect of the dorsum. Where the mucous membrane had been destroyed and replaced by fibrous tissue it contained numerous typical nodules. There was no pus. The disease was chiefly limited to the mucous membrane, with a few lesions placed superficially in the tongue muscle.

The submaxillary lymphatic glands were normal. The left retropharyngeal gland showed lesions of tuberculosis as well as the small shot-like nodules of actinobacillosis, while the right gland showed a typical cluster of actinobacillary nodules at its periphery.

Case B. 6.

A tongue was found on the slaughterhouse floor on June 23rd, 1929. On examination a number of typical nodules were found on each side of the dorsum.

One of the submaxillary lymphatic glands was slightly congested, the other appeared normal.

Case B. 7.

On July 2nd, 1929, the head of an Irish bovine animal, 18 months old, from which the tongue had been removed, was detected on a rack in one of the cooling rooms, by a member of the Meat Inspection staff.

Lesions.—The hard palate, particularly on the left side close to the cheek teeth, showed a large number of nodules, some of which had coalesced forming irregular areas, pale at the margins from the deposition of fibrous tissue. In a few instances the mucous membrane had been destroyed, leaving a red inflamed area showing numerous typical yellow nodules, the whole being surrounded by the raised edges of mucous membrane.

The hard palate showed many lesions similar to case B. 4.

The retropharyngeal glands were affected.

Case B. 8.

Subject.—A young Irish bullock, slaughtered on October 1st, 1929.

Lesions.—The substance of the liver contained a number of areas of fibrous tissue in which were embedded numerous small yellow, shot-like, actinomycotic nodules. There was no pus.

Part of the peritoneal covering of the liver and the adjacent peritoneum was inflamed and thickened and presented a typical actinomycotic appearance. The right xiphoid lymphatic gland showed a cluster of small nodules localised in part of the periphery, and was the only lymphatic gland presenting macroscopic lesions.

Case B. 9.

Subject.—A four-year-old Irish bullock in excellent condition, slaughtered on October 12th, 1929.

Lesions.—Head. The left retropharyngeal gland was affected in the usual way with small peripheral nodules. The right gland was tuberculous.

Tongue. A large dorso-lateral area on each side of the tongue showed typical lesions, but no ulceration.

Lungs. The right cardiac lobe contained numerous small actinomycotic areas from the size of a pea to a florin. When incised the cut surface was convex and in the smallest lesions the colour was yellowish, changing to a greenish tinge in the larger ones, while the largest of all showed radiating streaks of black pigment. The base of the right lung contained a large lesion which involved the diaphragm and part of the liver. When incised a small quantity of thick, yellowish-green pus oozed out and more could be expressed on pressure. The cut surface showed a great variety of colour, yellow, green, and black. The " abscess " was limited by a thick capsule, around which was an inflamed area containing a quantity of serum.

Cases B. 10 *and B.* 11.

During an examination of tongues in one of the offal rooms these two cases were detected. Both tongues were affected in the usual way on the dorso-lateral aspects. Case B. 10 showed numerous ulcerated areas.

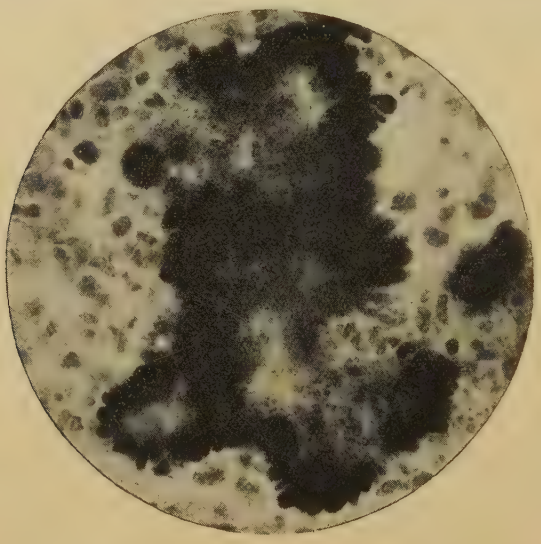

FIG. 2.

Section from tongue. Case B. 11. (× 800.) An actinobacillus colony showing large clubs (deeply stained) and bacillary centres.

Case B. 12.

This case when examined bacteriologically turned out to be one of tuberculosis, and it was quoted because macroscopically it was thought to be actino-bacillosis.

Subject.—A three year old Irish bullock in very good condition, slaughtered on October 27th, 1929.

Lesions.—Liver. the surface of the liver showed a number of circular

areas about the size of half a crown piece, yellowish-white in colour, and forming slight elevations on the surface of the liver. When the liver was sectioned a number of similar areas were seen throughout its substance. These areas when examined closely were found to consist of a matrix of white, glistening, fibrous tissue, rather irregular in outline, with a large number of small yellow nodules distributed throughout. There was no evidence of caseation or calcification, and no capsule around the lesions.

The portal lymphatic gland was enlarged and slightly paler than normal. It contained a number of small yellowish nodules arranged round the periphery of the gland, with a few clusters in parts.

Dorsal lymphatic glands. The last two glands of the dorsal chain showed one or two small nodules, which could only be detected on very close inspection.

In this case the other viscera and their associated lymphatic glands were normal.

Case B. 13.

Subject.—A large white Cheshire sow.

Lesions.—Udder. The udder presented a few small abscesses about the size of a hazel nut. When opened they gave exit to a non-offensive greenish pus.

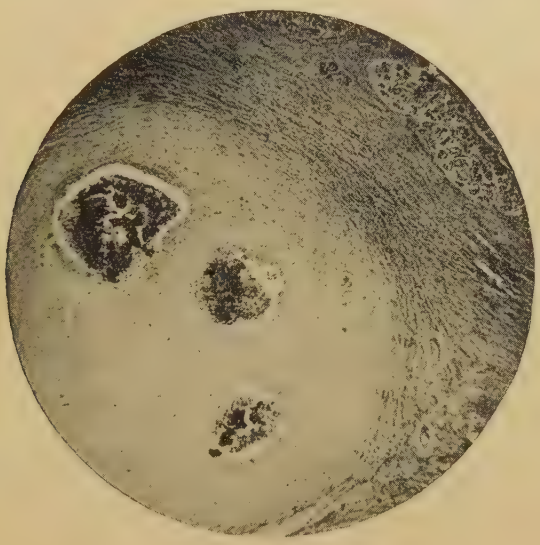

FIG. 3.

Section from mammary gland. Case B. 13. (× 25.) Shows comparatively large streptothrix colonies surrounded by fibrous tissue. (Compare with Fig. 1.)

In three of the anterior mammary glands there were six small indurated areas, each composed of a basis of fibrous tissue containing numerous small, yellow, hard, shot-like nodules. These small areas could only be detected by palpation of the udder, there being no external evidence of their presence.

Case B. 14.

Subject.—An Irish bullock, $3\frac{1}{2}$ years old, slaughtered on December 1st, 1929.

Lesions.—Lungs. The base of both lungs showed a very slight pleurisy on all surfaces. The thoracic lymphatic glands appeared normal.

Diaphragm. The serous coverings of the diaphragm were definitely inflamed, but the peritoneal covering was much more acute and extensive. The exudate was of the ordinary hæmorrhagic type undergoing organisation, like a simple non-specific inflammation of a serous membrane.

The peritoneum over the spleen and the pillars of the diaphragm were involved in the inflammatory process.

Rumen. The wall of the rumen was thickened from $\frac{1}{2}$ to $1\frac{1}{4}$-ins. over an area measuring 24-ins. long by 14-ins. at its broadest part.

The mucous membrane over the thickened part presented a number of blunt elevations, some of which were beginning to ulcerate. On section numerous very minute yellowish specks, almost undetectable by the naked eye, could be seen embedded in the mucous membrane and submucous tissue, which was $\frac{1}{3}$-in. thick. The cut surface was moist and pale.

The peritoneum on the abdominal wall in opposition to the lesion on the diaphragm was acutely inflamed, but, like that of the diaphragm, there was nothing actinomycotic in its character.

In this case the organism seemed to be limited to the wall of the rumen and the peritonitis in the neighbourhood was a simple inflammatory extension.

Case B. 15.

This case, like Case B. 12, was thought to be of an actinomycotic nature until a bacteriological examination was made, when it was found to be tuberculous.

Subject.—A three-year-old Irish bullock, slaughtered on December 8th, 1929.

Lesions.—Essentially a pleurisy and peritonitis, visceral and parietal membranes being affected.

The peritonitis was much more extensive, affecting practically the entire peritoneum with the exception of the pelvic membrane. The pleurisy was limited to the diaphragm and the base of both lungs.

There were three types of lesion present, *viz.*:—

1.—Cauliflower-like projections attached to the serous membrane by a delicate pedicle.

2.—Low convex growths.

3.—An even layer of an acute hyperæmic exudate. A close inspection of the cut surface revealed a number of minute yellowish points in the cauliflower and low convex growths.

There was no noticeable change in any of the lymphatic glands.

Case B. 17.

Subject.—A three-year-old Irish bullock, slaughtered on December 29th, 1929. In very good condition.

Lesions. Œsophagus. The œsophagus was affected from the point where it enters the thorax to the rumen.

The most striking feature was the extensive œdema of the gullet generally, but particularly of the submucous tissue, which contained a large quantity of yellow gelatinous semi-coagulated fluid.

Studded along the mucous membrane were a number of elongated, yellowish, indurated actinomycotic areas.

The submucosa was indurated in parts which presented numerous, minute, yellow points.

Parts of the muscular wall contained single hard nodules, some of which had become confluent.

The mediastinal lymphatic glands contained many typical nodules, situated mostly at the periphery.

Rumen. The wall of the rumen for several inches round the œsophageal opening was very much thickened and presented the appearance of numerous yellow spots, like millet seeds. The disease had extended along the wall of the rumen into the reticulum.

Liver. The substance of the liver contained a great many typical lesions consisting of areas composed of a basis of fibrous tissue in which were embedded numerous very small yellow points.

Case B. 18.

Subject.—A four-year-old Irish bullock in very good condition, slaughtered on December 29th, 1929.

Lesions.—Rumen. The wall of the rumen for a large area of about 14-ins. square was considerably thickened. The mucous membrane presented a few round hyperæmic projections.

The peritoneum over the thickened wall was inflamed but contained no actinomycotic lesions.

The lymphatic gland attached to the rumen showed numerous peripheral nodules, while one gland was affected throughout its substance.

Case B. 19.

Subject.—A three-year-old Irish bullock, in excellent condition, slaughtered on January 28th, 1930.

Lesions.—Hard palate. The left side of the hard palate in the region of the second, third, and fourth cheek teeth presented an ulcerated area measuring 3 by 10 cm.

The normal colour of the mucous membrane, which was black, had been destroyed by the ulcerative process, leaving the "floor" of the ulcer a light brown or fawn colour, slightly hyperæmic and granular looking.

When the soft tissue of the hard palate was removed and held between the observer and the light numerous very minute dark spots could be seen. When examined on the cut surface the spots presented the usual appearance of the small, yellow, shot-like actinomycotic nodules. See cases B. 4 and B. 7.

There were numerous nodules in the periphery of the left submaxillary lymphatic gland.

Case B. 21.

Subject.—A three-year-old Irish bull, slaughtered on February 16th, 1930.

Lesions.—The Reticulum. The affected area measured 30 by 22 cm. and the wall was 2 cm. thick. The appearance was as usual, small yellow nodules distributed throughout the thickened fibrous wall.

The peritoneal surface and part of the omentum was inflamed, moist, gelatinous, and at intervals there were little clusters of typical actinomycotic nodules.

A simple inflammatory process had extended through the diaphragm to the pericardium but no nodules were visible.

The most posterior ventral intercostal lymphatic gland was enlarged and contained one nodule.

PNEUMONIA OF BOVINES DUE TO *PASTEURELLA BOVISEPTICA*.

By Wm. Tweed, M.R.C.V.S., D.V.S.M., and
J. W. Edington, M.D., D.P.H.

I.

Clinical Report.

By Wm. Tweed, M.R.C.V.S., D.V.S.M.

Pneumonic lungs were being met with in the course of meat inspection at the Sheffield abattoir, and these exhibited lesions which differed markedly in appearance.

From the clinical histories of these animals I suspected that, contrary to prevalent opinion, some might be due to a specific organism, and thus decided to send all such lungs to Dr. Edington for pathological examination.

The Outbreaks.

(A) This was an outbreak of infectious pneumonia in a dairy herd.

Pneumonic lesions of a characteristic type were present in the lungs of a cow sent for emergency slaughter to the abattoir. Mr. S. E. Sampson, M.R.C.V.S., gave me the history of this cow, and it was found that she had been stalled No. 4 in Shed I, in a herd occupying three sheds. She had been ill two days, and showed symptoms so acute that slaughter was advised. Her lungs were sent for pathological examination as " Cow specimen No. 1."

Cow No. 3 in Shed I was also on this date showing symptoms of pneumonia, and the following day two more cows in stalls Nos. 2 and 6 in Shed II became affected with pneumonia.

Two days after, Nos. 3 and 4 in Shed II also contracted the disease, making a total of six animals affected out of 18.

No. 2 in Shed II was also slaughtered, and her lungs are classed as " Cow specimen No. 2 " in the pathological report.

The remaining four cows recovered. The outbreak occurred in the month of November, and the weather at that time was very damp and foggy.

(B) Cow specimen No. 3 was obtained from a newly calved cow sent for slaughter by a cattle dealer in the Bakewell area.

This animal was traced, and it was found that she had been purchased a few days previously in a cattle market and driven for some miles by road to this dealer's premises, where she developed pneumonia. The lungs showed lesions similar to those in specimens Nos. 1 and 2.

(C) Cow specimen No. 4 consisted of the lungs of a newly calved heifer from a farm adjoining that where outbreak (A) occurred. She had been stalled in a shed with nine other cows and was

immediately isolated and no other case occurred on these premises. This heifer died three days after the onset of symptoms, and was sent to a local knackery, where a post-mortem examination was made. The lungs showed the typical pneumonic lesions.

(D) Cow specimen No. 5 was discovered in the course of meat inspection in a cow slaughtered by a wholesale butcher in the abattoir. She had been bought in a large cattle market some considerable distance away, and was not traced further. Her lungs showed similar lesions to the previous specimens.

(E) Cow specimen No. 6 was obtained some months later from a cow again sent for slaughter by the cattle dealer in the Bakewell area. She had the same history as cow specimen No. 3 and showed the same lesions in the lungs.

(F) Cow specimen No. 7 was obtained from a Friesian bred cow from a herd of 14 animals, seven of which showed symptoms of pneumonia. This cow had an emphysematous swelling extending along the back which occurred about the fourth day after the onset of symptoms and disappeared a short time afterwards. No improvement was noted in her condition, and she was slaughtered. One other of the animals died, showing similar lesions in the lungs, and the remaining five recovered. A newly purchased animal entered this herd a few days before the outbreak, but it never showed any signs of the disease.

(G) This outbreak was reported to us by Mr. E. Marrison, M.R.C.V.S., Bakewell. Two calves were found dead on Mr. Marrison's first visit, and both had the typical pneumonic lesions in their lungs. This herd was visited by me, and it was found to consist of nine calves ranging in age from ten weeks to four months, 18 cows, and one bull. The calves were housed in a badly lighted and badly ventilated loose box surrounded on three sides by cowsheds, in which the cows were stalled. Communicating doors from this box opened into two cowsheds, and light and ventilation were inefficiently supplied by one small window on the open side.

On my visit a third calf was found showing symptoms of pneumonia and it was decided to slaughter it. Previous to this the animal was chloroformed, and the skin over the jugular vein was removed so that blood samples for blood culture could be taken under the most sterile conditions possible. The calf carcase with the organs intact was sent to the pathologist as " Calf specimen No. 1." This calf, which was ten weeks old, had only shown symptoms during the previous 24 hours.

Calf specimen No. 2 was obtained from a fourth calf which became affected and died. A post-mortem examination was made, and the lesions found corresponded with the previous specimens except that well marked bowel lesions were also present. Eventually the remaining five calves in this shed became affected. They all showed a rise in temperature and were coughing and exhibiting lung symptoms. They were put into a well ventilated shed and

with treatment recovered. They also supplied us later with blood samples for agglutination experiments.

Origin of Infection.

This is very doubtful.

Some predisposing causes will now be mentioned with notes on their possible effect in some of the above-mentioned cases.

1.—Journey by road or rail. This may act in two ways :—

(*a*) by lowering the resistance of the animal ;

(*b*) by bringing the animal into close contact with infection, *e.g.*, other animals or infected wagons, sheds, feeding and drinking troughs.

This factor may have been of importance in cases classed under the headings (B), (D), and (E).

2.—Period of the year. The disease is most prevalent during the winter months. This may be due to many causes, *viz.* :—

(*a*) Extremes, and especially rapid changes, of temperature, say from the warm cowshed to the much colder conditions experienced out of doors.

(*b*) Animals brought into closer contact.

(*c*) Bad ventilation and overcrowding.

(*d*) Generally worse sanitary conditions. This is well illustrated in outbreak (G) amongst calves, and may also have been of importance in outbreaks (A), (C), and (F).

3.—Another factor in the calf outbreak which may have had a predisposing influence was that the calves were being bedded with a supply of old, mouldy hay, which had been badly saved the previous year and was very dusty. The calves were seen eating this material.

4.—Introduction of a carrier of infection into the herd. This may have initiated the disease in outbreak (F), though the suspected animal was never proved to be a carrier.

Jones and Little (1921), in America, point out that animals newly introduced into a herd are often the first affected. This obviously is one side of the picture, as if a carrier be introduced into a non-immune herd the new animal will escape the disease whilst the others will become affected, whilst if a non-immune animal be introduced into an infected herd it will no doubt soon show symptoms of the disease.

According to Wooldridge (1923), the disease has been studied in the Argentine, United States, Philippine Islands, and India, as well as in Europe, but the existence of the disease in the British Isles has not been established. He also states that young animals are more susceptible than old to both natural and artificial infection. This was noted in the calf outbreak, in which all the animals in the shed contracted the disease.

Symptoms.

The earliest symptom is a rise in body temperature, reaching 105° F. to 108° F., followed by cessation of feeding and depression. The respiration becomes accelerated, then greatly hurried, and

the cough is hard and suppressed. The animal stands with its head extended, and later becomes recumbent with the head still advanced. The mucous membrane of the nostrils is injected, and a greyish, often blood-stained, discharge exudes. Breathing becomes so difficult that this discharge is not removed by the animal's tongue.

Pleurisy is often present, and the animal grunts when moved, or after coughing or lying down. Examination of the chest reveals consolidation of lung tissue and in some cases fluid in the pleural cavity. The disease may run an acute course for three or four days, followed by death, or the animal may commence to feed and recovery slowly take place. The cough persists for some time, and the temperature slowly falls to 103° F., or lower, but may remain irregular for some time.

In one case, viz., Cow specimen No. 7, emphysema under the skin of the back was well marked, but in none of the outbreaks so far met with have œdematous lesions or intestinal symptoms been noted before death.

In the calf outbreak symptoms of the mixed forms of the disease were anticipated, but death took place from the pectoral form. It was, therefore, evident that we were dealing with the pectoral form of hæmorrhagic septicæmia.

Diagnosis.

The disease has to be differentiated from other pneumonic conditions, viz., the onset of miliary tuberculosis and tubercular pneumonia. The onset of pleurisy is soon evident, and as a rule the pneumonia develops rapidly.

The history of the admission to the herd of a newly purchased animal recently exposed to infection and of other of the above-mentioned predisposing factors may assist in diagnosis. If more than one animal becomes affected with pneumonia in the herd, and if tuberculosis can be excluded, the disease should be suspected. In countries where the disease is prevalent three forms of hæmorragic septicæmia may occur separately or in mixed form, viz., intestinal, pectoral, and œdematous. In the intestinal form the animal shows symptoms of restlessness and colic with diarrhœa. The lesions of this form were present in combination with the pectoral form in the calf outbreak described above, but no symptoms of intestinal disease were noted. This form may follow the other forms of the disease if the lung symptoms are very acute and the onset of septicæmia is rapid. The symptoms of this variety of the disease and of bracken poisoning in cattle have several points in common.

The diseases associated with the shipment and transit of animals which usually make their appearance a few days after the animals reach their destination are of great importance (J. M. Buchanan, 1929). Of these hæmorrhagic septicæmia is one which requires further research work, especially as to whether the animals in their condition of lowered vitality pick up the infection while in transit or the organisms are present normally in some animals and only infect them when their resistance is sufficiently lowered.

Post-Mortem.

The lesions in the lungs can be distinguished quite readily from other forms of pneumonia seen in bovines, so that a collection of those showing typical lesions can easily be made.

The lesions in the seven cows' lung specimens described in this report can therefore be described in general as follows:—

The lungs were greatly enlarged. The pneumonic areas were clearly demarcated from the non-pneumonic areas, the latter portions being pale pink in colour and spongy. The lung surface varied in colour from the pink of the healthy lung to the dark red of the hepatised areas.

The interlobular tissue was very marked and distended, due chiefly to the presence of coagulated exudate.

Distinct stages of pneumonia were often present in the same lung. Healthy lobules appeared between the consolidated ones. The pneumonic areas were usually confined to the lower borders of the lungs, but the whole of the apical lobe was commonly involved.

The cut surface of the pneumonic area of the lung showed a striking picture of consolidated tissue interspersed with thickened septa. The dark red of the hepatized lung intersected by the thick white septa gave the diseased tissue a mottled appearance. The appearance is very similar to that seen in contagious pleuro-pneumonia of bovines.

The cut surface of the consolidated areas was moist and exuded a serous fluid. In the more chronic cases grey circular dry areas could be seen amidst the solid tissue. The pleura was thickened, and usually had a greyish fibrinous deposit on its surface. Fluid was present in the pleural cavity.

The mucous membrane lining the trachea and smaller air passages was inflamed and showed a blood-stained exudate mixed with air. In some of the cases the pericardial sac contained fluid and was adherent to the surrounding lung tissue. Petechial hæmorrhages were scattered over the heart surfaces both externally and internally, and pericarditis was often present.

The liver was slightly congested in all cases.

The spleen sometimes showed petechiæ under its peritoneal covering.

The kidney was generally enlarged, but occasionally showed reddened areas on its external surface.

With the exception of Cow specimen No. 4, no inflammation of the intestines was noted in any of the cows. In this case there was inflammation of the fourth stomach and small intestines. In the others congestion was present, this being most marked in those showing the most acute lung lesions.

In most cases emphysema was present in the fatty tissue surrounding the kidneys and tissues of the back. This, however, is met with in other types of bovine pneumonia.

In the calf outbreak the disease was in a very acute form. The two first animals which died showed typical pleuro-pneumonia with

excess of pleuritic fluid and whitish fibrinous deposits on both pleural surfaces. This gave the appearance of pustular material which might easily have led one to describe the condition as septic pleuro-pneumonia.

Petechial hæmorrhages were found scattered throughout the muscles and serous membranes of the body, and blood spots were seen along the jugular furrows and under the scapulæ. These were present in the connective tissue and muscles of those areas.

The fourth stomach, the small intestines, and particularly the duodenum, were inflamed. In the duodenum the mucous membrane was ulcerated.

In the third calf, which was killed by chloroform, the disease was in its early stage, and the lesions in the lungs showed chiefly congestion with hæmorrhages into some of the lobules. A few of these, however, were commencing to hepatise. Petechiæ were present in the serous membranes of various organs, *e.g.*, in the pericardium. No bowel lesions were present in this calf.

The post-mortem on the fourth calf which died showed an acute pleuro-pneumonia. The lungs were adherent in places to the chest wall, with greyish fibrinous exudate on the pleural surfaces. Both lungs were almost completely hepatised and liver-like in colour and consistency. The pleural cavities contained some blood-stained fluid.

The pericardium was adherent to the lungs and an acute pericarditis was present.

The fourth stomach and small intestines were inflamed, and blood-red circular areas about a quarter-inch in diameter were present on the leaves of the third stomach and on the mucous membrane of the fourth stomach and duodenum. Ulceration was commencing in the fourth stomach and duodenum. Petechiæ were also present in the connective tissue and muscle along the jugular furrows, and these hæmorrhages in the connective tissue seemed to follow the course of the large blood vessels.

II.

Pathological Report.

By J. W. Edington, M.D., D.P.H.

My clinical colleague, having noticed that pneumonias, other than tuberculous, in bovines in England were not as uncommon as previously thought, decided to send me the diseased organs from such animals with a view to the identification of the cause.

As the investigation proceeded it was found that the cases could be divided into two groups, typical examples of which could easily be recognised on macroscopic examination, viz. :—

(A) Where the lungs were markedly consolidated and the interlobular septa thickened.

(B) Where the lungs showed patches of broncho-pneumonia, with areas of collapse and marked emphysema of the interlobular septa. The bronchi of the affected areas were usually plugged with a thick tenacious mucopurulent secretion.

The present investigation deals with group A.

Pathological Examination.

The following is a description of the material received for examination :—

From Cow 3 and Cow 4 : portion of one lung.

From Cow 1, Cow 2, Cow 5, Cow 6, and Cow 7 : both lungs.

From Calf 1 and Calf 2 : both lungs, and various other organs.

From this it is obvious that a thorough examination could only be carried out in the case of one organ, the lung. The lungs, however, appear to bear the brunt of the infection in the majority of cases.

The Lungs.

The appearance of the pneumonic lungs is very characteristic. The diseased lobules are of a liver-like consistence, and of a deep red colour. Small grey areas can be seen throughout the red, and these are most marked towards the centre of the lobule. Thus at first sight the lobule appears to have a grey centre with a deep red periphery.

A striking effect is obtained when a healthy lobule is found in an otherwise pneumonic portion of lung. It appears as a pale pink spongy area in the midst of deep red consolidated tissue.

If a portion of the hepatised lung be squeezed a large amount of blood-stained fluid can be obtained. This high fluid content gives rise to an interesting appearance if such a lung be cut across and left overnight at room temperature. During this period a clear reddish-yellow fluid exudes, and sets into a clear jelly of the same colour. The surface of the lung resting on the table is lightly embedded in this, whilst globules of the jelly appear on the free surface, especially along the thickened septa.

Examination of Sections.

In early cases, especially in those rapidly fatal ones of septicæmic type, there is marked congestion of the blood vessels in the alveolar walls, with occasional hæmorrhages into the alveoli. This is followed later by an exudation of serum into these spaces, with a desquamation of the lining alveolar epithelium.

Still later the alveoli become packed with polymorphonuclear and catarrhal cells. This is the stage when the typical macroscopic picture is obtained.

The bronchi of the diseased area show varying grades of inflammation, and are often plugged with pus cells and their disintegration products.

Many of the blood and lymph vessels are filled with coagulated material. This is best seen in the interlobular septa, and is probably

one of the causes of the thickening of the septa and also of the high fluid content of the consolidated lung. In cases which recover the pneumonic lung may undergo complete resolution, but occasionally chronic abscess formation may occur. In the latter the animal becomes a carrier of the infection and, discharging as it does infected sputum, is a source of danger to other animals.

A fibrinous pleurisy was present in some of the cases.

In two cases, Calf 1 and Calf 2, some of the other organs were examined.

Liver.

This was extremely congested, and there was cloudy swelling of the liver cells. In Calf 2 some of these cells had undergone a fatty change.

Spleen.

This organ also showed intense congestion. A good deal of blood pigment was present, some of which was lying loose in the sinuses, whilst some had been phagocytosed by large mononuclears.

Kidney.

There was marked congestion of the kidney, with cloudy swelling of its parenchymatous cells. In Calf 2 there was also a commencing tubular nephritis.

Alimentary Tract.

In Calf 1 the fourth stomach and the small intestines were greatly congested. In Calf 2 there was in addition ulceration of the fourth stomach. On examining an ulcerated area the submucosa was found to be very œdematous, with great dilatation and thrombosis of its blood and lymph vessels.

Blood Vessels.

A characteristic appearance of the disease in both of the calves was the presence of small hæmorrhages in the connective tissues. This was especially well seen in the fatty connective tissue lying along the auriculo-ventricular groove of the heart.

Bacteriological Examination.

In all the cases the causal organism was the same, and was easily isolated from the consolidated lung, where it abounded quite unmixed with other organisms.

In the severe rapidly fatal cases (Calf 1 and Calf 2) the bacillus was obtained from the blood stream ; but in the typical pneumonic cases (Cow 2, Cow 4 and Cow 6) blood cultures were invariably negative.

Morphology.

In the tissues the organism appears as a small bipolar bacillus.

Smears from the consolidated lung demonstrate this excellently, especially if Leishman's stain be used. The bacillus stains readily with the ordinary aniline dyes and is Gram-negative.

It is non-motile, asporogenic, and measures from 0·5 μ to 2·0 μ in length. On culture, however, much longer forms can be obtained. Short chain formation can sometimes be seen in broth cultures.

Cultural Characteristics.

This organism grows well on agar, and shows a decided preference for moisture. Thus it grows luxuriantly on agar which has been overheated in preparation and is consequently soft and very moist.

After 24 hours' growth on agar at 37° C. a single colony has a characteristic appearance, being markedly lenticular and clear, with a strikingly glistening surface. As the colony ages it becomes more opaque and somewhat umbonate. The margin is entire and the outline circular.

When the surface growth is collected into a heap it has a yellowish tint with the slightly waxy consistence of lemon curd. The colour deepens with age.

After about a week's incubation at 37° C. the growth develops a slight stickiness, and later, when the medium has become somewhat dehydrated, it is firmly adherent.

In broth it grows readily, producing at first a turbidity and later a deposit and a tendency to pellicle formation. This deposit is granular and does not adhere to the inoculation loop.

Other suitable media for its propagation are boiled blood agar, blood agar, and Cole's tryptamine agar.

It grows on McConkey's bile salt agar, does not liquefy gelatine, and does not produce indole.

It reduces nitrates, produces acid in milk, gives a negative Voges Proskauer reaction, and is not bile soluble. Some strains produce a slight hæmolysis in blood agar (horse, rabbit, and human blood were used in strengths of 6 to 8 per cent.). The fermentation results were as follows :—

Acid and no gas were produced with glucose, galactose, lævulose, saccharose, mannite, maltose, lactose, sorbite, and xylose ; but neither acid nor gas with mannose, adonite, amygdalin, dextrin, starch, dulcite, erythrite, inulin, isodulcite, salicin, and raffinose. The effect of the different strains on inosite, glycerine, and arabinose was variable.

Three strains (Cow 6, Calf 1 and Calf 2) after prolonged subculture showed slight fermentation of mannose. In the same way strains Cow 7 in inosite ; Cow 4 and Cow 7 in glycerine ; Cow 3, Cow 4, Cow 7 and Calf 2 in arabinose, which showed no acid production when first isolated did so after long subcultivation.

Agglutination Characteristics.

All the organisms were proved by agglutination and absorption of agglutinins to belong to Group 1 (Jones) of *Pasturella boviseptica.*

In the study of this type of pasturella other organisms of the hæmorrhagic septicæmia group were examined. The following is a list of all the organisms studied with their histories :—

Cow 1.—From a small epidemic which was reported by Messrs. S. E. Sampson and W. Tweed in the " Veterinary Record " of March, 1928, vol. viii, page 235.

Cow 2.—Also from the above epidemic.

Cow 3.—Obtained from a newly calved milch cow in a cattle dealer's premises in the Bakewell area.

Cow 4.—From a newly calved heifer from a farm adjacent to that from which strains Cow 1 and Cow 2 were obtained.

Cow 5.—From a case discovered during meat inspection at the abattoir. History unknown.

Cow 6.—From a milch cow from the cattle dealer's premises at Bakewell. Obtained six months after strain Cow 3 had been isolated.

Cow 7.—From a Friesian cow killed at the abattoir.

Calf 1 *and Calf* 2.—Both strains were obtained from an epidemic amongst ten to sixteen weeks' old calves at a farm near Baslow.

P. boviseptica (Group 1).—Calf 1339. Kindly sent me by Dr. Jones, Rockefeller Institute, Princeton, U.S.A.

P. boviseptica (Group 3).—929, National Collection of Type Cultures M.R.C. Originally from Dr. Houghton, Parke Davis Co., Detroit, U.S.A. (01448). Used in the preparation of sera in bovine hæmorrhagic septicæmia.

P. Boviseptica (group 3-1287) National collection of Type cultures. Originally from Dr. Jones from a case of necrotic pneumonia in a calf (Bov. Pn. 1). This is one of the two strains which formed Group 3 in Dr. Jones' paper (1921).

P. boviseptica (Group 3).—Cow 1177 and Cow 981. Both strains were kindly sent me by Dr. Jones.

I have been unable to get an authentic strain of Group 2. Certain other strains producing hæmorrhagic septicæmia in animals other than bovines were obtained. Those with numbers attached were received from the National Collection of Type Cultures.

P. cuniculicida. 1550. Isolated from Belgian hares during an epizootic.

P. avicida. 121. Pasteur Inst. II. From fowl cholera.

P. oviseptica. 1877. From Dr. Houghton. (08366.) Used in the preparation of sera in ovine hæmorrhagic septicæmia.

P. suiseptica. 1875. From Dr. Houghton. (98368.) Used in the preparation of sera in porcine hæmorrhagic septicæmia.

P. suiseptica. " Pig." History unknown.

" Mouse." 948. Isolated by Dr. Topley from a mouse during an epizootic.

" Rat Kangaroo." 1898. Isolated by Dr. N. Lucas from a caseous lesion in the lung of a rat kangaroo.

" Cat." 2771. Isolated by Prof. T. Hare Roy. Vet. College, London, from the heart blood of a domestic cat.

" Tiger." 2827. Isolated by Col. Hamerton and Dr. Lovell from the pneumonic lung of a tiger.

" Rat." 2764. Isolated from a rat by Dr. K. F. Mayer, California, U.S.A.

The following is a comparative study of the above-mentioned strains :—

Morphology.

As described above for the organisms of Group 1 of *P. boviseptica*. There are no distinguishing features.

Cultural Characteristics.

All the strains, except those belonging to *P. boviseptica* (Group 1), produced very mucoid deposits after a week's growth in broth at 37° C. The deposit could be readily lifted *en masse* on an inoculation loop. In the case of strains of *P. boviseptica* (Group 1) the deposit was granular and did not adhere to the loop.

Biochemical Characteristics.

These can be seen at a glance by consulting Table 1. It will be observed that all the strains produce acid, but no gas in glucose, galactose, lævulose, saccharose, mannite, and sorbite, and with the exception of strains *P. boviseptica* (Group 3, 01448), rat, and rat kangaroo, in xylose.

Organisms of *P. boviseptica* (Group 1) are the only ones which do not produce acid in mannose. They also produce acid in lactose and maltose, and occasionally in arabinose, inosite, and glycerine.

Acid is not formed from these substances by the other strains, with the following exceptions :—

P. boviseptica (Group 3, 01448) from arabinose, and the rat kangaroo strain from lactose.

With the exception of *P. cuniculicida* in dulcite, none of the organisms produce acid in adonite, amygdalin, dextrin, starch, dulcite, erythrite, inulin, isodulcite, salicin, and raffinose. After a week's active growth at 37° C., the final H ion concentration in the tubes showing an acid reaction is between *p*H 6·0 and 6·5. Two sugars, lactose and arabinose, show less active acid production, especially with recently isolated strains, the final H ion reading being from *p*H 6·8 to 7·0.

Tubes not showing acid production usually demonstrated increased alkalinity, the H ion reading being from *p*H 7·8 to 8·2.

(The sugar media had as a basis fermented bullock's heart broth. The substances to be tested were used in a 1 per cent. concentration. All were sterilised by filtration through a Seitz filter, with the exception of inulin and starch, which were sterilised by steaming at 100° C. for twenty minutes on three successive days.)

Production of Indole.

All the strains, except those belonging to *P. boviseptica* (Group 1), produce indole.

(Bacto tryptophane broth was used.)

Growth on McConkey's Bile Salt Agar.

None of the strains, except those of *P. boviseptica* (Group 1), grow on this medium.

(The medium was made as described in Eyre's Bacteriological Technique, 2nd ed., page 205.

The method of testing the organisms was as follows :—

A McConkey's agar plate was inoculated, and after three days' ærobic incubation at 37° C. another plate of the same material was inoculated from it. This in turn was incubated ærobically for three days at 37° C., and then examined for the presence of growth.)

Bile Solubility.

None of the organisms are bile soluble.

As *P. boviseptica* (Group 2) is said by Jones (1921) to be bile soluble, this feature should be of great value in its recognition.

(The test was carried out both with ox bile and 2 per cent. sodium taurocholate. The method used is described in Muir and Ritchie's Manual of Bacteriology, 8th ed., page 272.)

Voges Proskauer Reaction.

All the strains produce a negative reaction.

Reduction of Nitrates.

All the strains give definite reduction.

(These last two tests were carried out as described in Muir and Ritchie's Manual of Bacteriology, 8th ed., page 400.)

Litmus Milk.

P. boviseptica (Group 1) organisms and the rat kangaroo strain are the only ones which produce acid in milk. All the others show no obvious change.

(The results were read after a fortnight's ærobic cultivation at 37° C.)

Gelatine.

None of the strains liquefy gelatine.

Hæmolysis.

None of the strains produce hæmolysis in blood broth after incubation for two days at 37° C.

On blood agar plates, whether surface spread or shake cultures, none of the organisms with the exception of some strains of *P. boviseptica* (Group 1) produce hæmolysis after 24 hours' ærobic incubation at 37° C.

After this period, however, all the organisms commence to show a wide spreading hæmolysis, which becomes very marked by the end of three days. This is probably due to the production of metabolic products other than a true hæmotoxin, but requires further investigation. Jones (1921) mentions that *P. boviseptica* (Group 1) strains

TABLE I.

Organisms.	*Glucose.*	*Galactose.*	*Levulose.*	*Saccharose.*	*Mannite.*	*Sorbite.*	*Xylose.*	*Arabinose.*	*Lactose.*	*Maltose.*	*Mannose.*	*Inosite.*	*Glycerine.*	*Salicin.*	*Isodulcite.*	*Dulcite.*
Cow 1	+	+	+	+	+	+	+	+	+	+	—	+	—	—	—	—
Cow 2	+	+	+	+	+	+	+	+	+	+	—	+	—	—	—	—
Cow 3	+	+	+	+	+	+	+	—	+	+	—	+	—	—	—	—
Cow 4	+	+	+	+	+	+	+	—	+	+	—	—	—	—	—	—
Cow 5	+	+	+	+	+	+	+	+	+	+	—	+	+	—	—	—
Cow 6	+	+	+	+	+	+	+	+	+	+	—	+	+	—	—	—
Cow 7	+	+	+	+	+	+	+	—	+	+	—	—	—	—	—	—
Calf 1	+	+	+	+	+	+	+	+	+	+	—	+	+	—	—	—
Calf 2	+	+	+	+	+	+	+	—	+	+	—	+	+	—	—	—
P. boviseptica (1) (Calf 1339)	+	+	+	+	+	+	+	+	+	+	—	+	+	—	—	—
P. boviseptica (3), (Cow 981).	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
B. boviseptica (3), (Bov. Pn. 1).	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
P. boviseptica (3), (01448)	+	+	+	+	+	+	—	+	—	—	+	—	—	—	—	—
P. boviseptica (3), (Cow 1177B).	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
Mouse (807A)	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
Cat (5985)	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
Pig (Unknown)	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
Tiger (2827)	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
P. cuniculicida (550) ...	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	+
P. oviseptica (1877) ...	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
P. suiseptica (1875) ...	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
P. avicida (121)... ...	+	+	+	+	+	+	+	—	—	—	+	—	—	—	—	—
Rat (2764)	+	+	+	+	+	+	—	—	—	—	+	—	—	—	—	—
Rat Kangaroo (1898) ...	+	+	+	+	+	+	—	—	+	—	+	—	—	—	—	—

In the Fermentation Tests.
+ = Acid, but no gas.
— = No acid or gas.

In Milk.
Ac. = Acid production.
Unc. = No change.

In the Other Tests.
+ = Positive reaction.
— = Negative reaction.

TABLE I (*Continued*).

Organisms.	*Adonite.*	*Amygdalin.*	*Dextrin.*	*Starch.*	*Erythrite.*	*Inulin.*	*Raffinose.*	*Milk.*	*Indole.*	*Growth on McConkey Agar.*	*Blue Solubility.*	*Voges Proskauer Reaction.*	*Hæmolysis.*	*Reduction of Nitrates.*	*Liquefaction of Gelatine.*
Cow 1	—	—	—	—	—	—	—	Ac.	—	+	—	—	—	+	—
Cow 2	—	—	—	—	—	—	—	Ac.	—	+	—	—	—	+	—
Cow 3	—	—	—	—	—	—	—	Ac.	—	+	—	—	—	+	—
Cow 4	—	—	—	—	—	—	—	Ac.	—	+	—	—	—	+	—
Cow 5	—	—	—	—	—	—	—	Ac.	—	+	—	—	—	+	—
Cow 6	—	—	—	—	—	—	—	Ac.	—	+	—	—	+	+	—
Cow 7	—	—	—	—	—	—	—	Ac.	—	+	—	—	+	+	—
Calf 1	—	—	—	—	—	—	—	Ac.	—	+	—	—	+	+	—
Calf 2	—	—	—	—	—	—	—	Ac.	—	+	—	—	+	+	—
P. boviseptica (1), (Calf 1339)	—	—	—	—	—	—	—	Ac.	—	+	—	—	+	+	—
P. boviseptica (3), Cow 981)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
P. boviseptica (3), (Bov. Pn. 1)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
P. boviseptica (3), (01448) ...	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
P. boviseptica (3), (Cow 1177B)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
Mouse (807A)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
Cat (5985)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
Pig (Unknown)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
Tiger (2827)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
P. cuniculicida (1550)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
P. oviseptica (1877)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
P. suiseptica (1875)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
P. avicida (121)...	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
Rat (2764)	—	—	—	—	—	—	—	Unc.	+	—	—	—	—	+	—
Rat Kangaroo (1898)	—	—	—	—	—	—	—	Ac.	+	—	—	—	—	+	—

In the Fermentation Tests.
+ = Acid, but no gas.
— = No acid or gas.

In Milk.
Ac. = Acid production.
Unc. = No change.

In the Other Tests.
+ = Positive reaction.
— = Negative reaction.

produce an early hæmolysis. I have noticed this with strains *P. boviseptica* (Group 1, Calf 1339), Cow 5, Cow 6, Cow 7, Calf 1 and Calf 2 ; but not with Cow 1, Cow 2, Cow 3 and Cow 4.

(In carrying out the hæmolysis tests human, horse, and guinea-pig blood were used. The blood agar contained 6 to 8 per cent. of either citrated or defibrinated blood.

All the organisms were tested in blood broth, and also by means of surface and shake blood agar plates.)

Agglutination Tests.

All the organisms, Cow 1, Cow 2, Cow 3, Cow 4, Cow 5, Cow 6, Cow 7, Calf 1, Calf 2, and *P. boviseptica* (Group 1, Calf 1339) were found by cross agglutination and absorption of agglutinins experiments to belong to Group 1 of *P. boviseptica*. The agglutinating sera were prepared from rabbits.

The sera used in these tests were made from strains Cow 1, Cow 2, Cow 6, Calf 1, and *P. boviseptica* (Group 1, Calf 1339). The only trouble experienced was the inagglutinability of the Cow 3 strain when first isolated. This was overcome by daily subculture in broth for three weeks.

Such *P. boviseptica* (Group 1) sera do not agglutinate Group 3 organisms or any of the other pasturella strains obtained from other animals ; neither do they agglutinate *B. typhosus*, *B. paratyphosus A*, *B. paratyphosus B*, *B. enteritidis* (Gaertner), *B. ærtrycke* (Mutton), *B. ærtrycke* (Newport), *B. dysenteriæ* (Shiga), *B. dysenteriæ* (Sonne), *B. dysenteriæ* (Flexner), nor *B. abortus* (Bang). From the biochemical reactions it can be seen that the so-called Group 3 of *P. boviseptica* resembles very closely the pasteurella strains obtained from other animals.

After examining the cross agglutination and the absorption of agglutinins of four Group 3 strains, viz. : 01448, Bov. Pn. 1, Cow 1177, and Cow 981, it was found that they showed little antigenic relationship. Table 2 shows a cross agglutination experiment illustrating this.

These strains have also little antigenic relationship with the other pasturella organisms as is seen in Table 3.

Table II.

Organisms of P. Boviseptica, *Group* 3.	*Sera of* B. Boviseptica, *Group* 3.			
	01448.	*Bov. Pn. I.*	*Cow* 1177.	*Cow* 981.
01448...	1,000	Nil	Nil	150
Bov. Pn. I	50	5,000	Nil	Nil
Cow 1177	Nil	Nil	3,000	150
Cow 981	Nil	50	Nil	1,000

Results of a Cross Agglutination Experiment with Four Members of *P. Boviseptica*, Group 3.

TABLE III.

Sera of P. boviseptica, *Group* 3.	*Other Pasturella Organisms.*										
	Cat.	*Pig.*	*Tiger.*	*Rat.*	Cuniculi	Ovisept.	Suisept.	Avicida.	*Mouse.*	*Rat Kangaroo.*	*Homologous Strain.*
01448	—	—	—	—	50	—	—	—	—	—	1,000
Bov. Pn. 1	50	—	—	50	—	—	—	125	—	—	3,000
Cow 1177	—	125	250	25	—	—	—	—	—	25	1,000
Cow 981	—	—	—	—	—	—	—	—	—	—	1,000

Agglutination of Various Animal Strains of Pasteurella with *P. boviseptica* Group 3 Sera.

Presence of Agglutinins in Bovines which have Suffered from a Group 1 P. boviseptica Infection.

The blood sera of 100 cattle killed at the Sheffield abattoir were tested with a *P. boviseptica* (Group 1) emulsion. This was the Cow 1 strain, and it was highly agglutinable (about five times more agglutinable than the average organism).

The results were as follows :—

Sera agglutinating to a titre of	0		=	2·0	per cent.
,, ,, ,,	1 in	25	=	4·0	,,
,, ,, ,,	1 in	50	=	36·0	,,
,, ,, ,,	1 in	125	=	34·0	,,
,, ,, ,,	1 in	250	=	22·0	,,
,, ,, ,,	1 in	500	=	1·0	,,
,, ,, ,,	1 in	1,000	=	1·0	,,

All these agglutinins could be entirely absorbed with either a Group I or a Group 3 *P. boviseptica* with the exception of the serum whose titre was 1 in 1,000. A Group I organism could easily absorb the agglutinins in this serum, but a Group 3 organism only partially did so, *i.e.*, after exhaustive absorption with a Group 3 the serum still agglutinated a Group 1 organism to a titre of 1 in 125.

On this evidence it is presumed that of the 100 animals examined one at least had previously suffered from a *P. boviseptica* (Group 1) infection.

A series of 50 calves were examined in a like manner with similar results except that not one showed any evidence of a previous infection with *P. boviseptica* (Group 1). This was in accord with the life history of these animals.

Five sera from calves which had recovered from a known *P. boviseptica* (Group 1) infection were examined. The blood was removed from these animals about three weeks after recovery.

They agglutinated a *P. boviseptica* (Group 1) as follows :—

Calf A = 1 in 50 : Calf B = 1 in 250 : Calf C = 1 in 500 ;
Calf D = 1 in 500 ; Calf E = 1 in 250.

All these agglutinins could be absorbed with a *P. boviseptica* (Group 1) organism ; but after absorption with a Group 3 *P. boviseptica* the sera still agglutinated a Group 1 organism as follows :—

Calf D = 1 in 250 ; Calf E = 1 in 50.
Calf A = 1 in 25 ; Calf B = 1 in 125 ; Calf C = 1 in 250 ;

This illustrates the presence of specific agglutinins for *P. boviseptica* (Group 1) in the blood serum of these calves.

Pathogenicity of P. boviseptica.

Many inoculations, intravenous, intraperitoneal, and subcutaneous, were made with all the Group 1 strains into rabbits, and these, with one exception, produced no obvious disease. The exception was in the case of the Cow 4 strain. The first subculture of the latter in broth was inoculated intravenously into a rabbit, the dose being about

10,000 millions, and the animal died within two days from septicæmia caused by the injected bacilli.

In the early stages of the investigation the possibility of the disease being contagious pleuro-pneumonia was considered. To decide this, the lung juices from one of these animals were filtered through a Seitz filter and then inoculated intravenously into a healthy calf. The animal showed no signs of ill-health. Cultures were also made from the same lung from which the filtered juices had been obtained, and a *P. boviseptica* (Group 1) was isolated in pure culture. This primary agar culture was suspended in normal saline solution and inoculated intravenously into a healthy calf, the dose being about 50,000 millions. The animal died in 36 hours from a *P. boviseptica* (Group 1) infection. A striking feature at the autopsy was the presence of numerous small hæmorrhages in the subcutaneous tissues.

This experiment was repeated with similar results.

The Group 3 *P. boviseptica* is much more virulent to rabbits than is the Group 1.

In the preparation of Group 1 agglutinating sera living cultures could generally be used with impunity. If, however, similar dosage was tried with a Group 3 organism the rabbit invariably succumbed.

Summary.

1.—All the cases of bovine pneumonia belonging to Group A which were examined were due to an infection caused by *Pasturella boviseptica* belonging to Jones' Group 1.

2.—The differential characteristics of use in the separation of this organism from others of the pasturella group are as follows :—

Tests.	P. boviseptiea. *Group* 1.	*Other Pasteurellæ.*
After one week's growth at 37° C. in broth.	Granular deposit.	Thick mucoid deposit.
Growth on McConkey's agar.	Growth.	No growth.
Milk.	Acid.	Most strains no acid.
Indole.	Negative.	Positive.
Hæmolysis.	Some strains positive.	Negative.
Fermentation differences.	Acid in lactose and maltose, but not in mannose.	Acid in mannose, but not in lactose or maltose.
Agglutination.	Agglutinated by a *P. boviseptica* Group 1 serum.	Not agglutinated by a *P. boviseptica* Group 1 serum.
Relative virulence.	Non-virulent to rabbits.	Group 3 *P. boviseptica* is virulent to rabbits.

It is with pleasure that I thank Dr. Jones, of the Rockefeller Institute, Princeton, U.S.A., for sending me various strains of his groups of *Pasturella boviseptica*.

I also wish to thank the North Midland Veterinary Association for their kindness in purchasing calves for the experimental work.

Conclusion.

There is a type of bovine pneumonia in England which is due to infection with a *Pasturella boviseptica* belonging to Jones' Group 1.

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REVIEW.

Some Diseases of Farm Animals. Bulletin No. 1, Ministry of Agriculture and Fisheries, 5th Edition. London : 1930. Price 1s. 6d. To be obtained from the Ministry, 10 Whitehall Place, London, S.W.1.

This Bulletin takes the place of the series of separate leaflets previously published by the Ministry in order to provide stockowners with the knowledge that they ought to possess regarding the diseases of farm animals, especially those of which notification is required under the Contagious Diseases of Animals Acts. It embodies information, accurate and up-to-date, regarding 26 diseases, and contains 18 helpful illustrations. The work reflects great credit on the veterinary staff of the Ministry, and deserves to have a very wide sale among farmers and other owners of stock.

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